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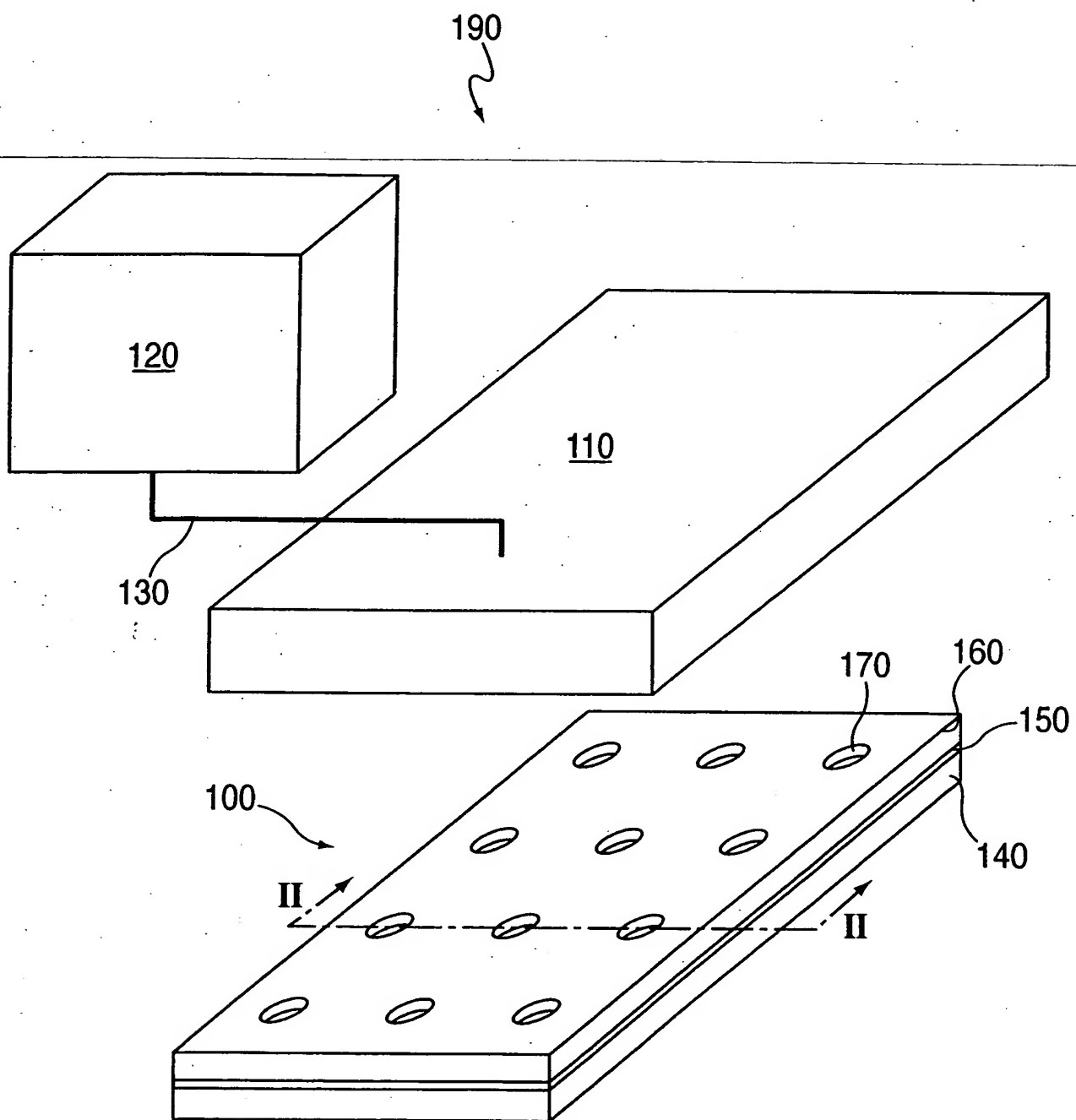


FIG. 1a

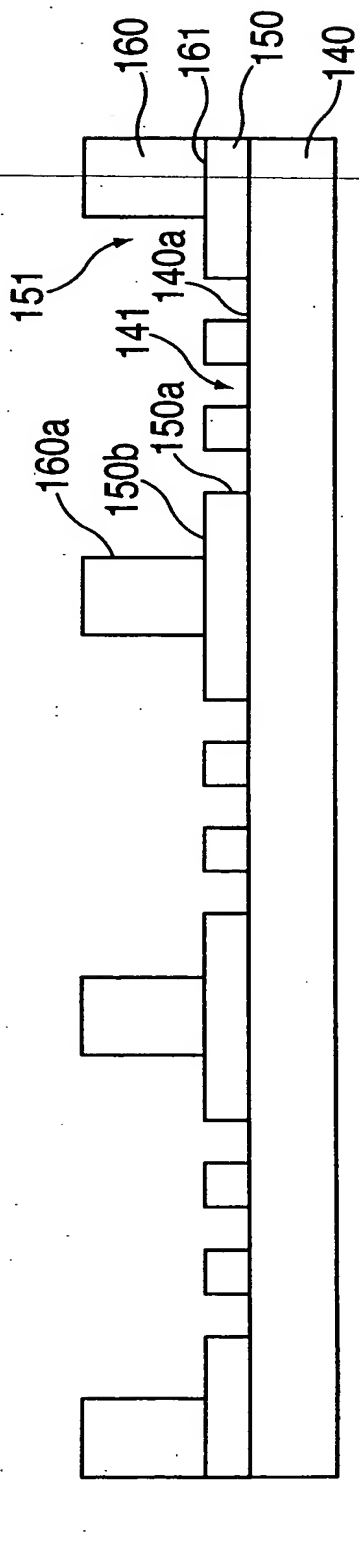


FIG. 1b

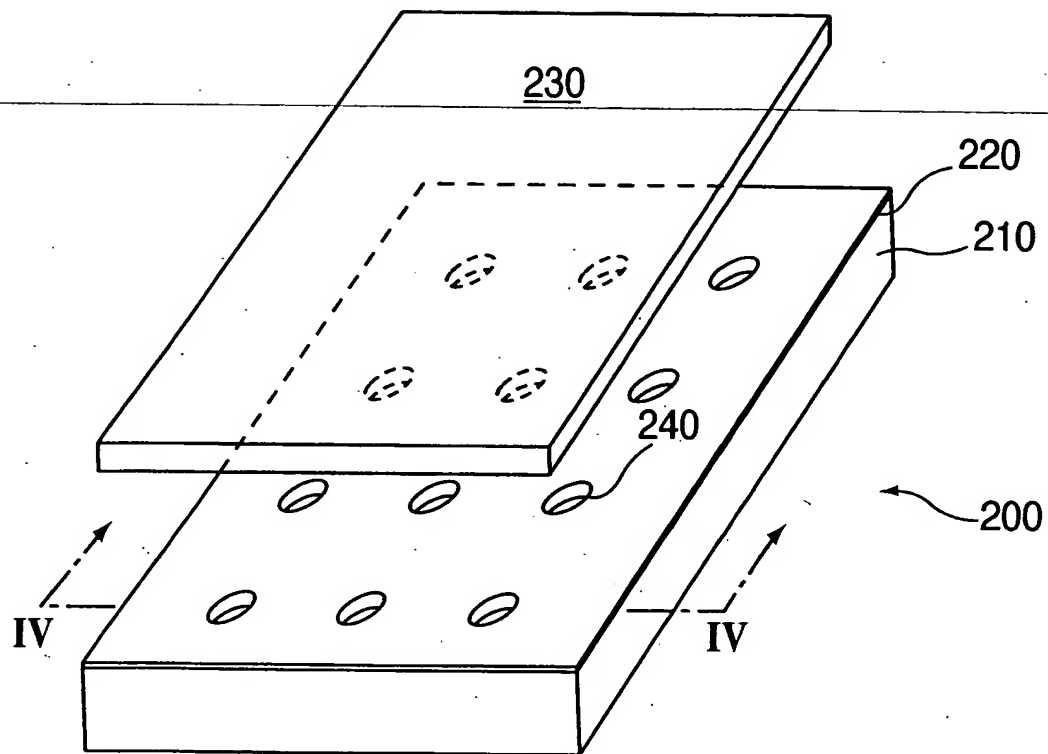


FIG. 2a

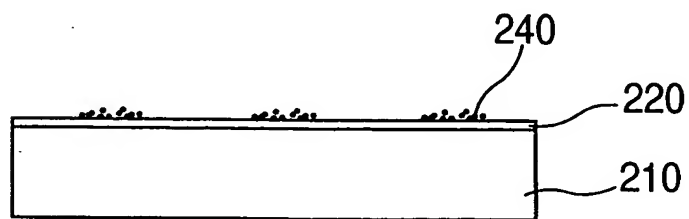
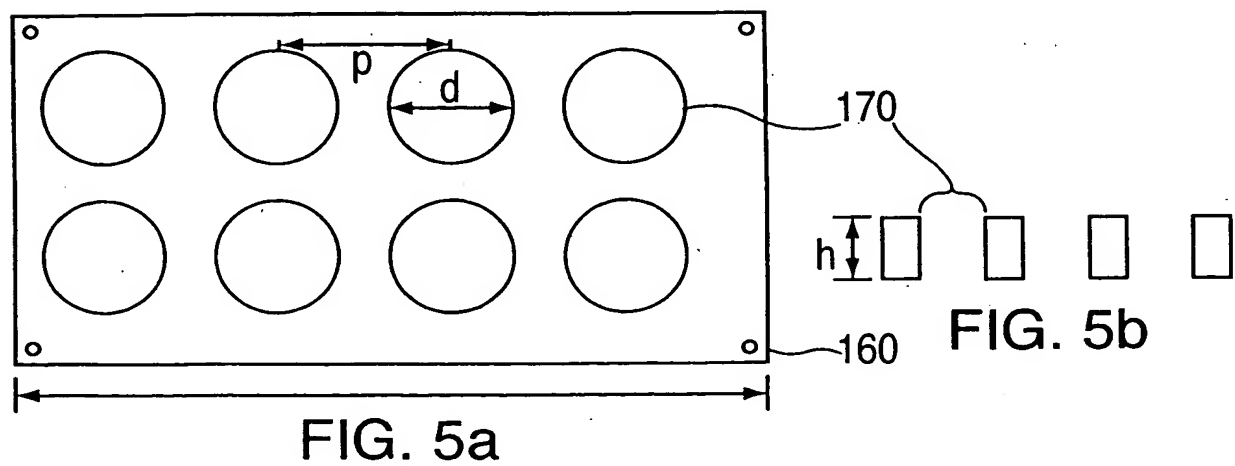
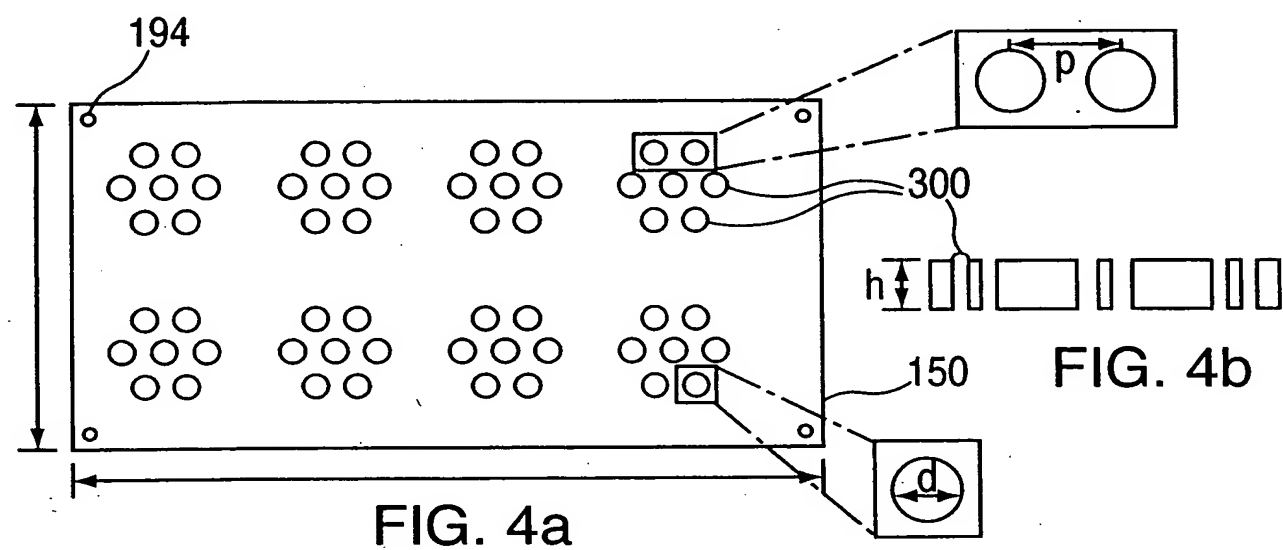
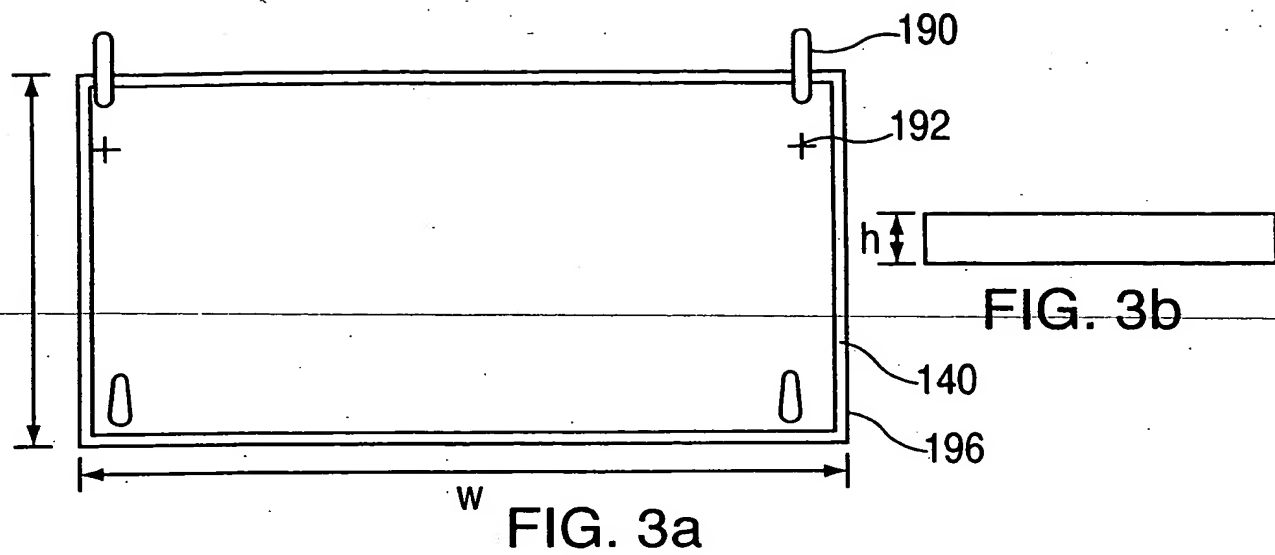
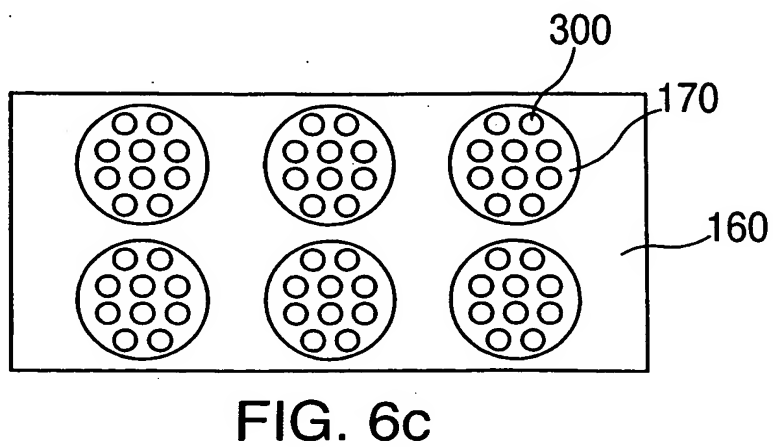
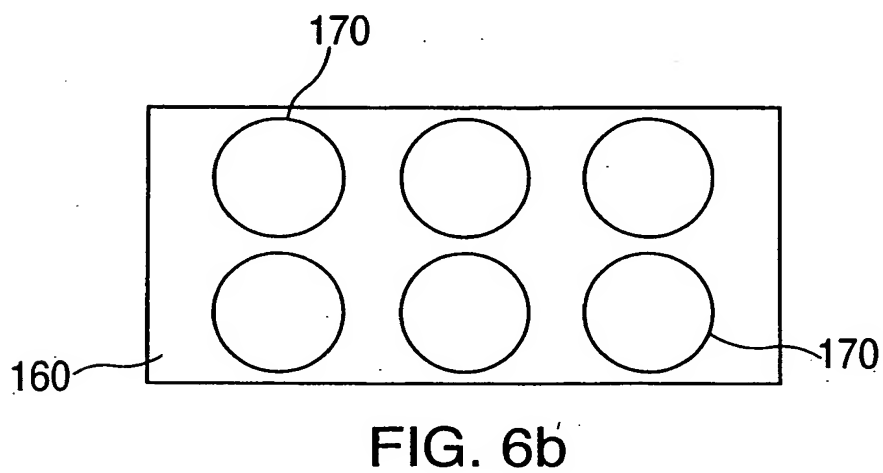
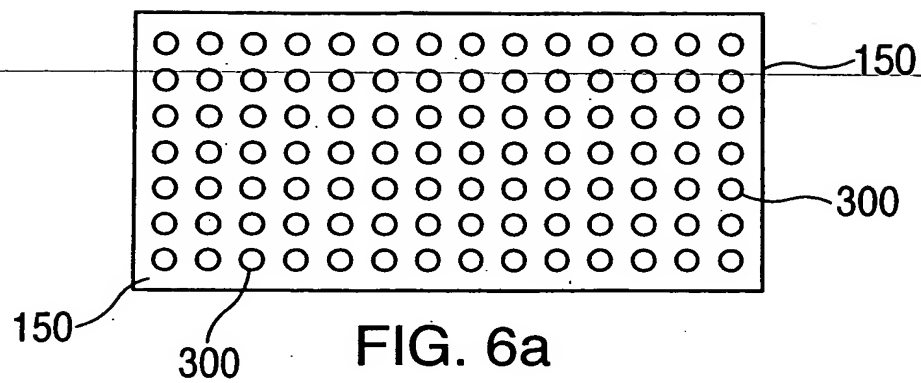


FIG. 2b





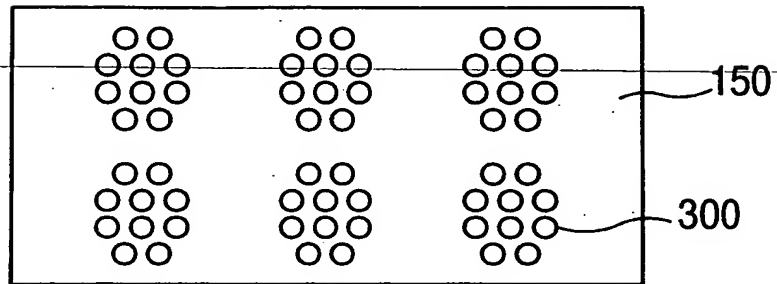


FIG. 7a

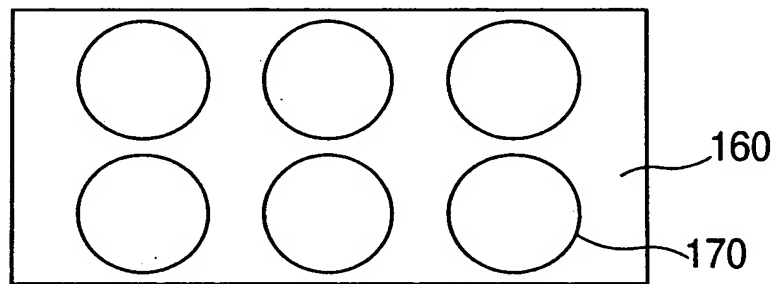


FIG. 7b

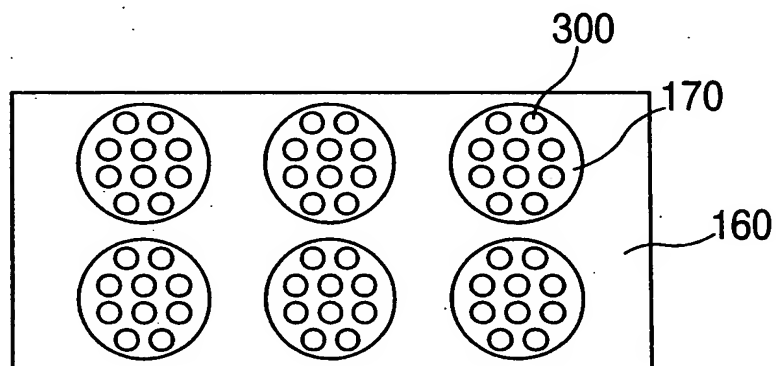
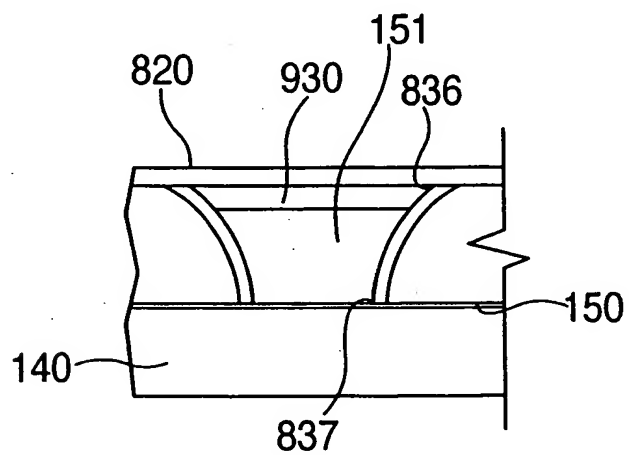
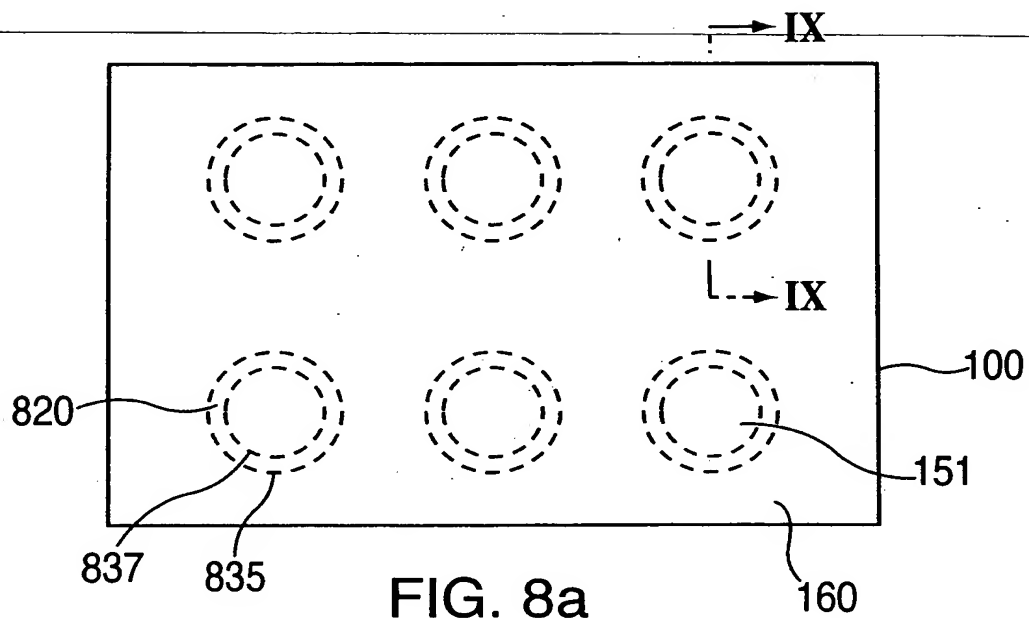


FIG. 7c



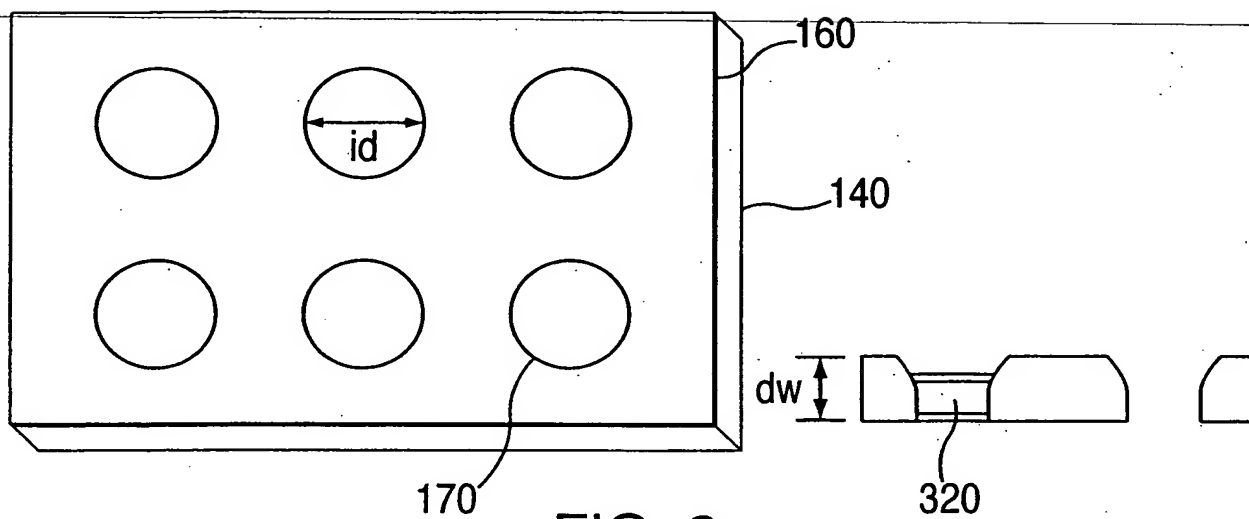


FIG. 8c

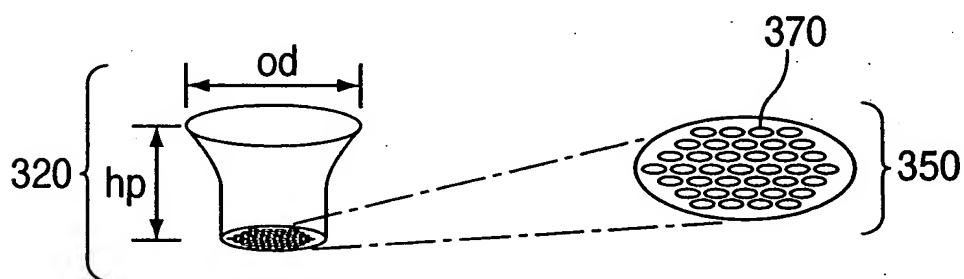


FIG. 8d

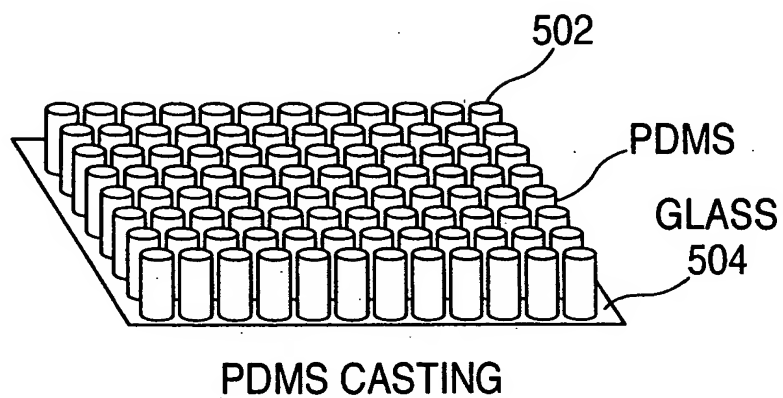


FIG. 9a

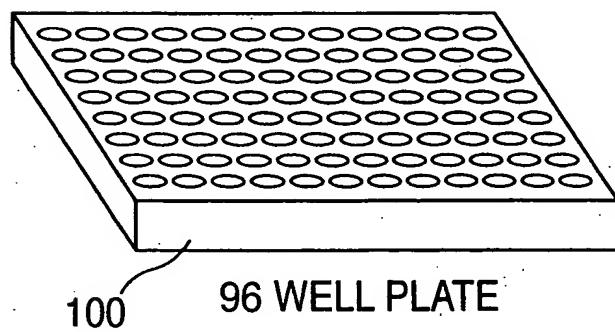


FIG. 9b

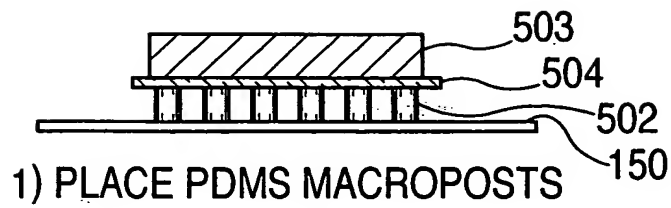


FIG. 10a



FIG. 10b



FIG. 10c

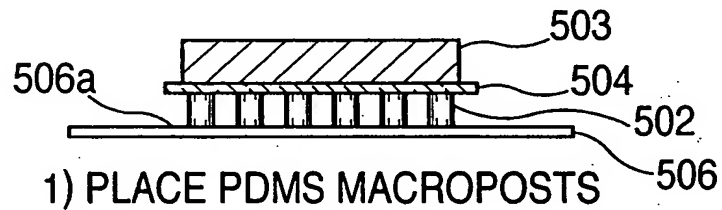


FIG. 11a

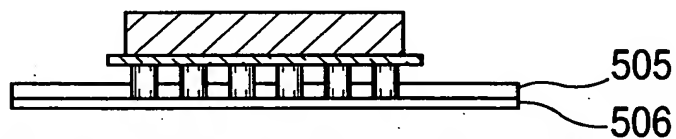


FIG. 11b



FIG. 11c

MS1
(ENDOTHELIAL)

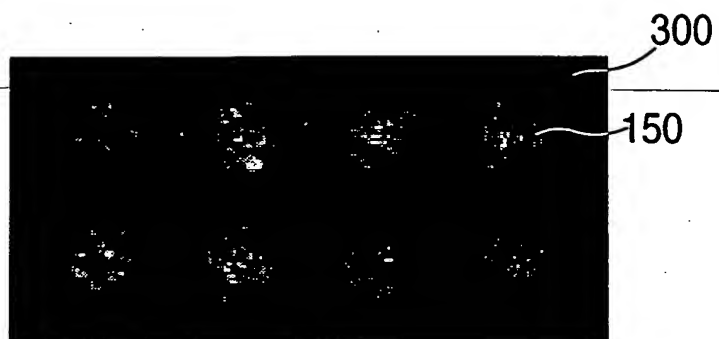


FIG. 12a

3T3
(FIBROBLAST)

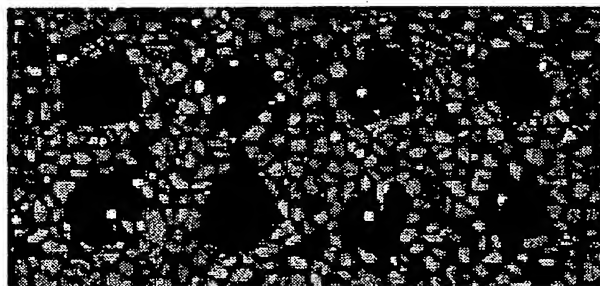


FIG. 12b

OVERLAY

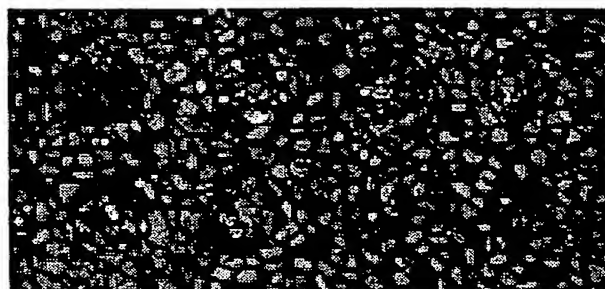


FIG. 12c

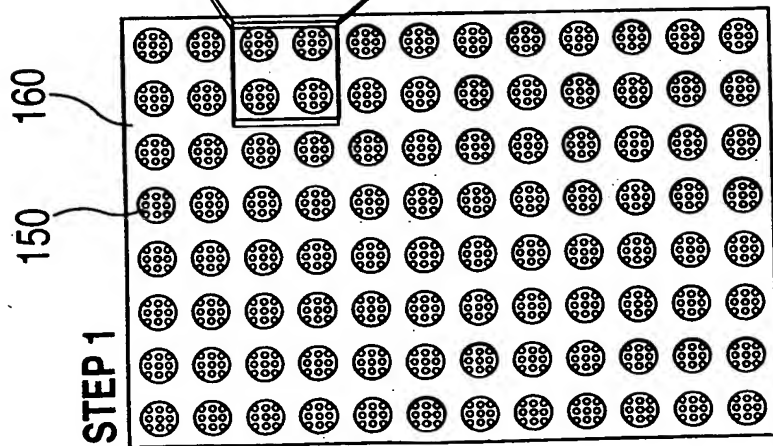


FIG. 13a

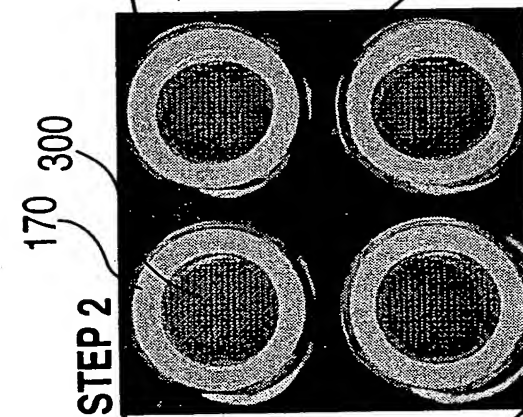


FIG. 13b

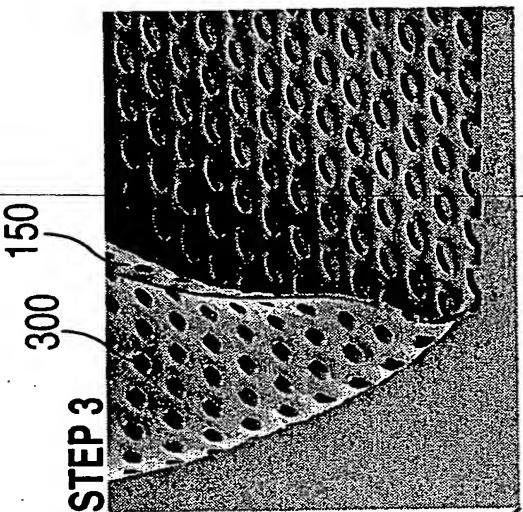


FIG. 13c

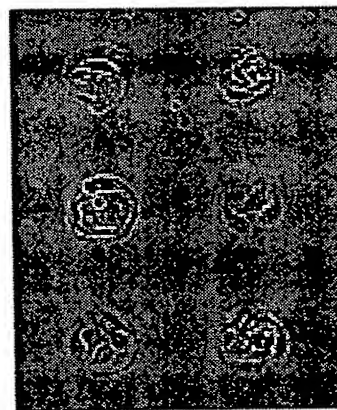


FIG. 13d

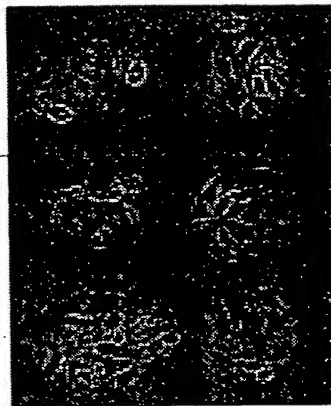
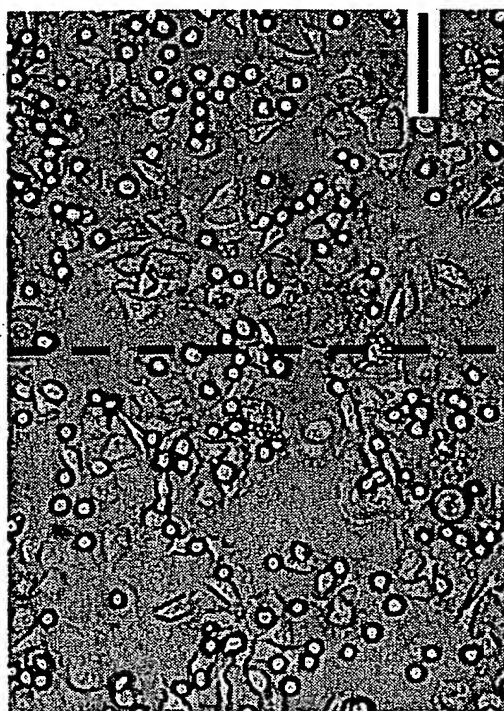
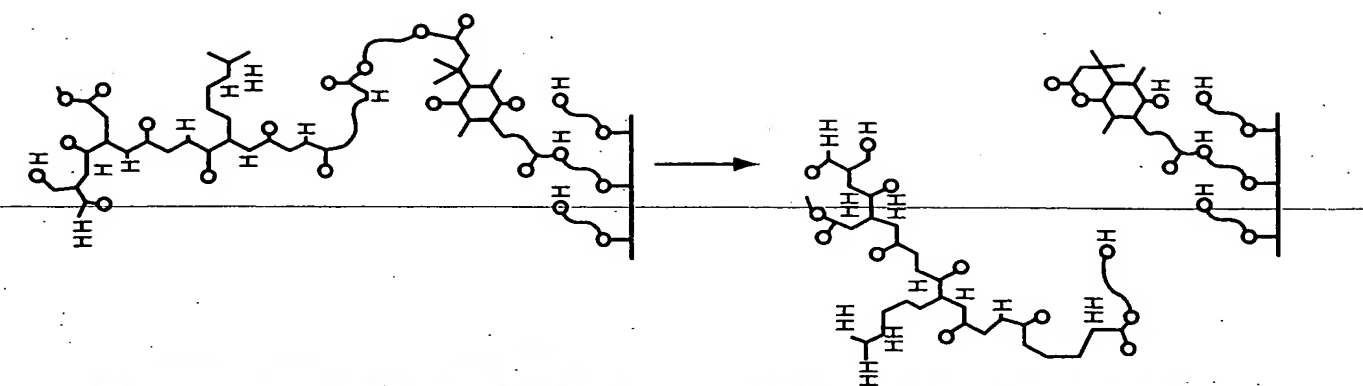


FIG. 13e



APPLY ELECTROCHEMICAL POTENTIAL

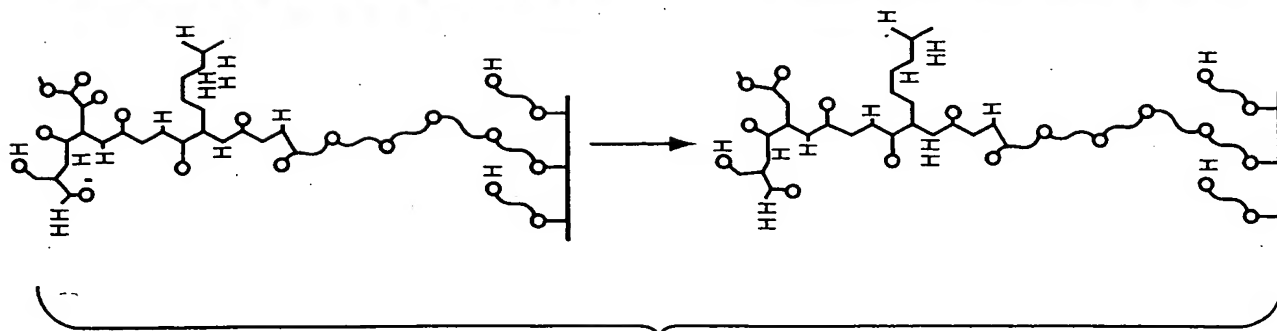
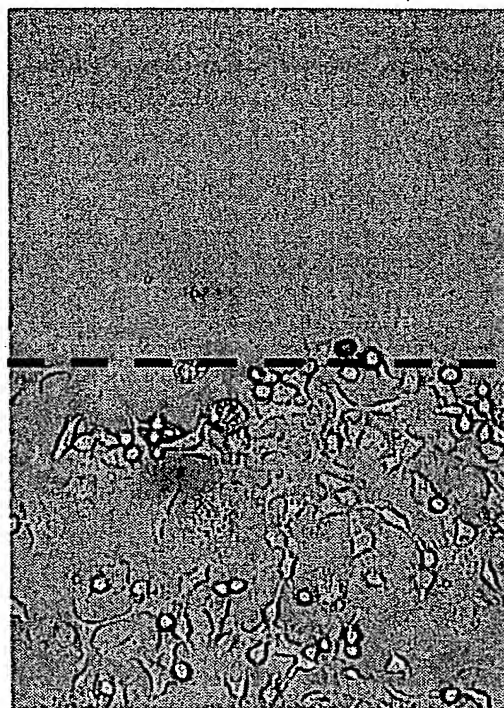


FIG. 14

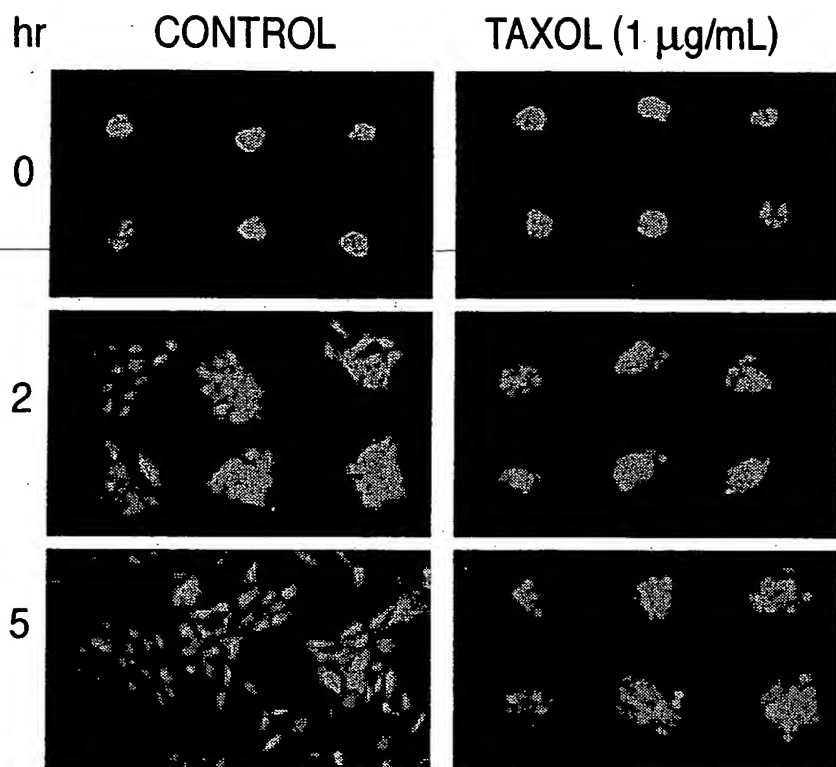


FIG. 15a

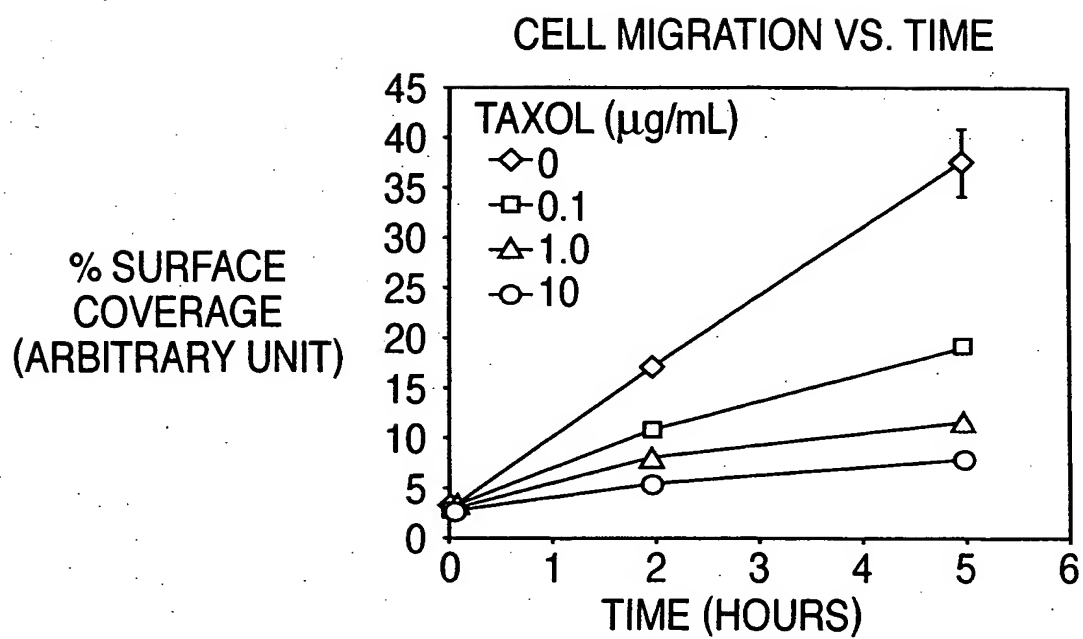


FIG. 15b

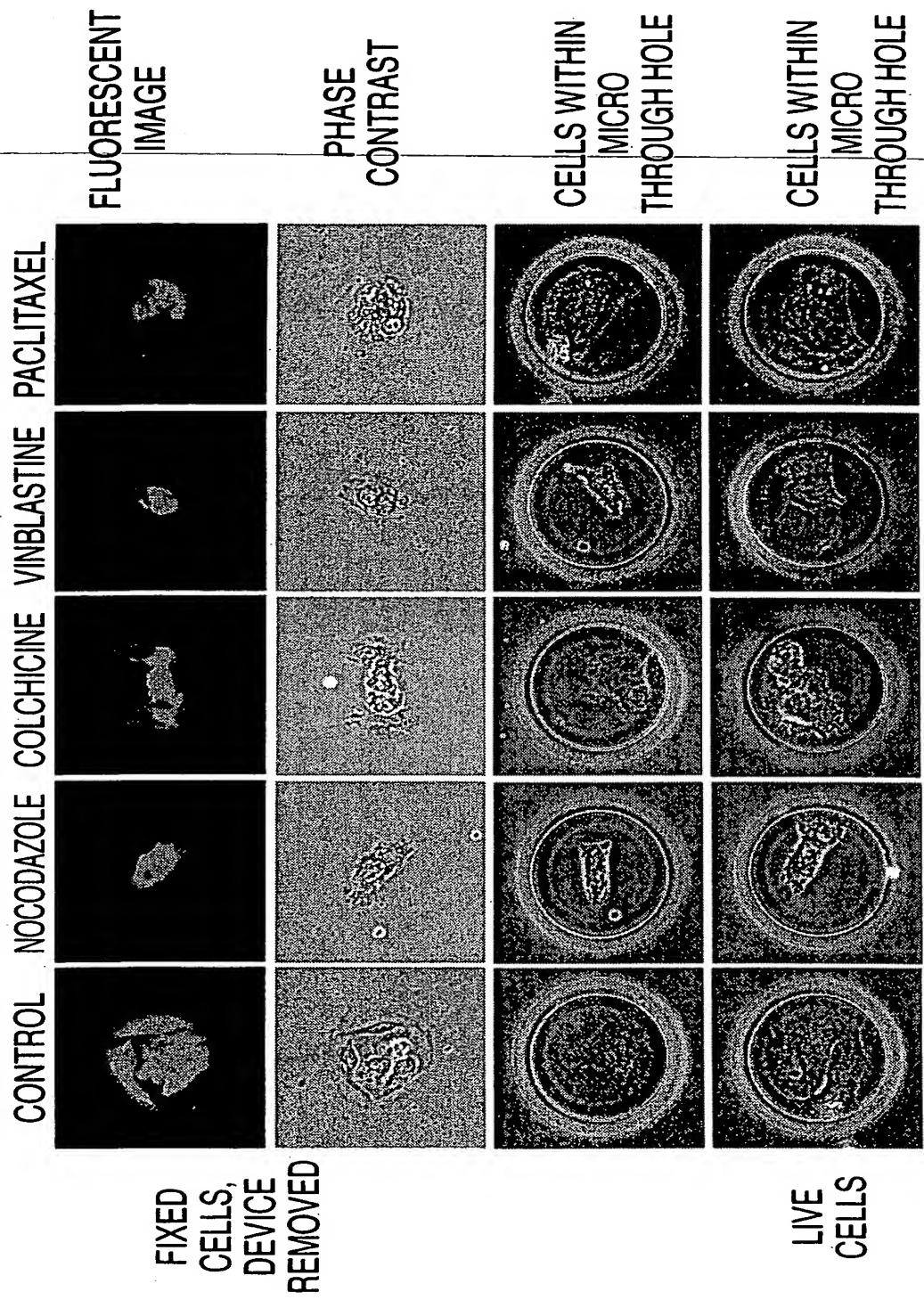
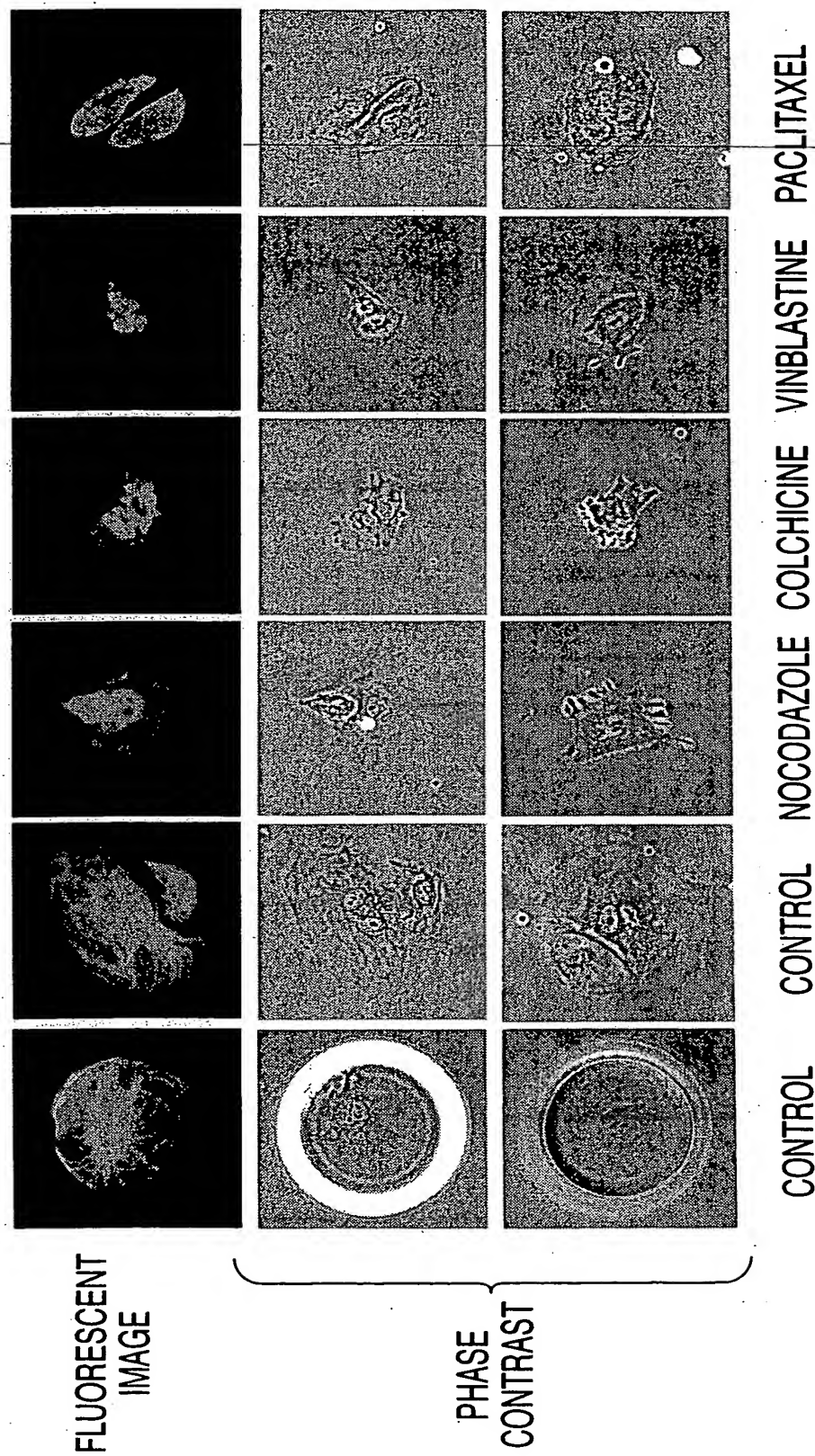


FIG. 16



DRUGS ADDED JUST PRIOR TO REMOVAL
OF PATTERNING MEMBER

FIG. 17

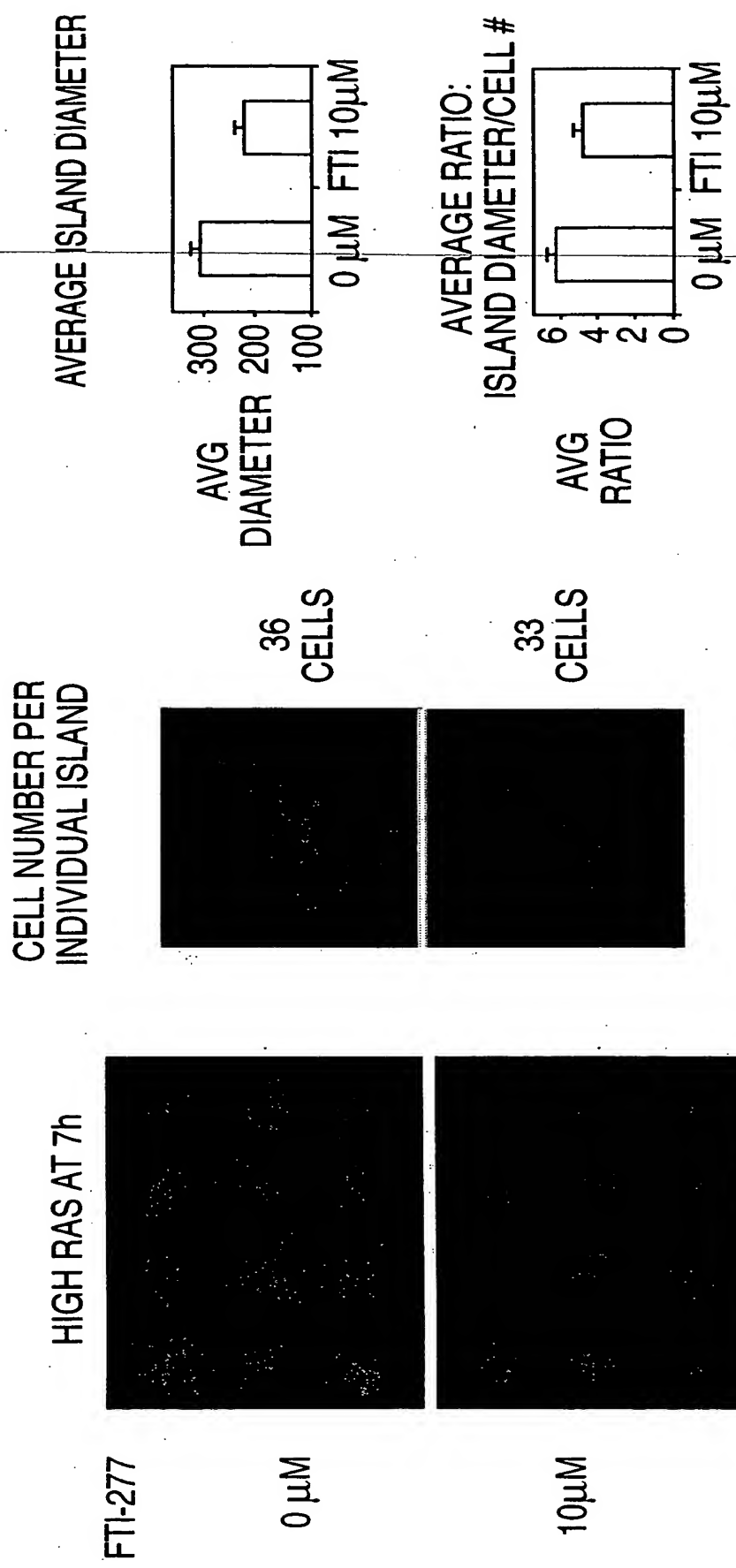


FIG. 18

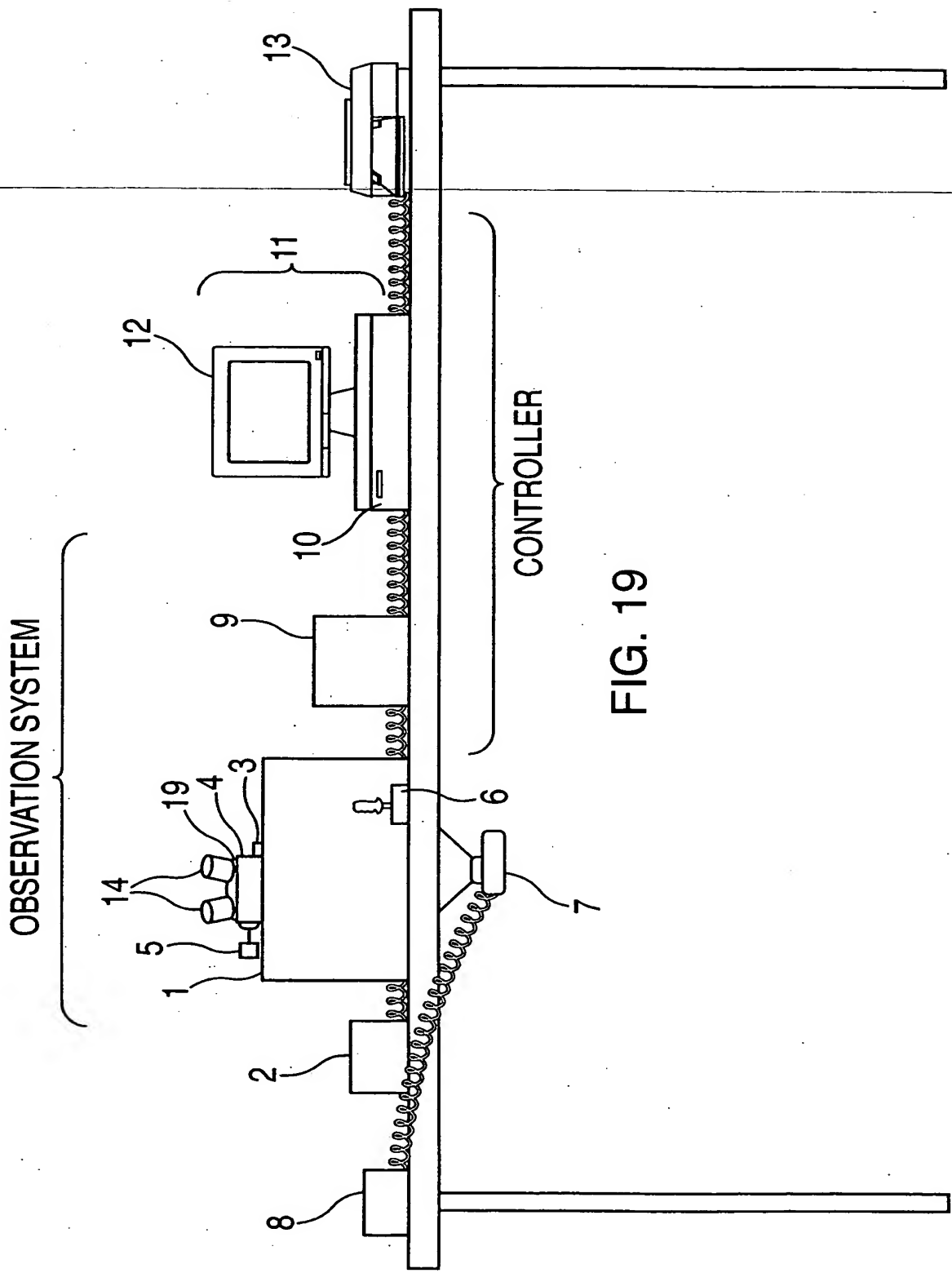


FIG. 19

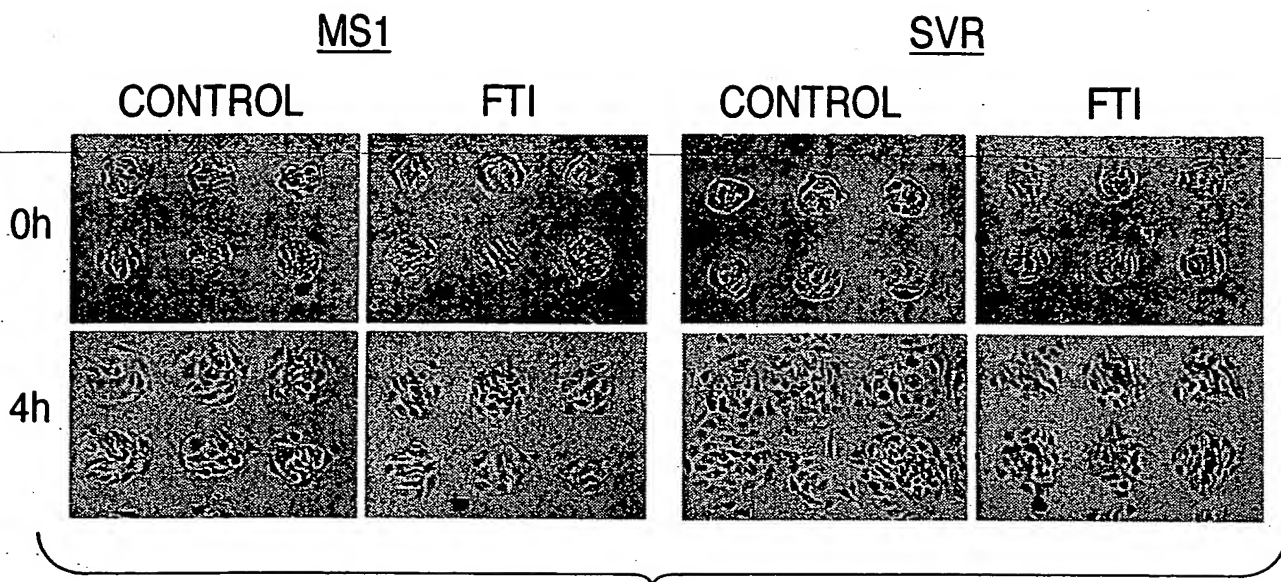


FIG. 20

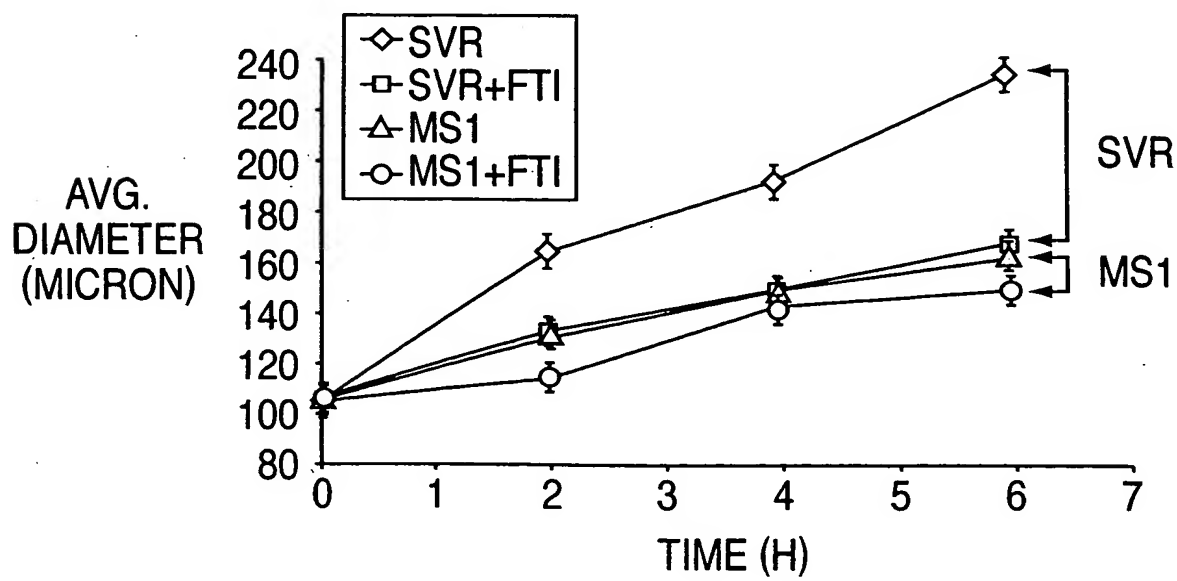


FIG. 21

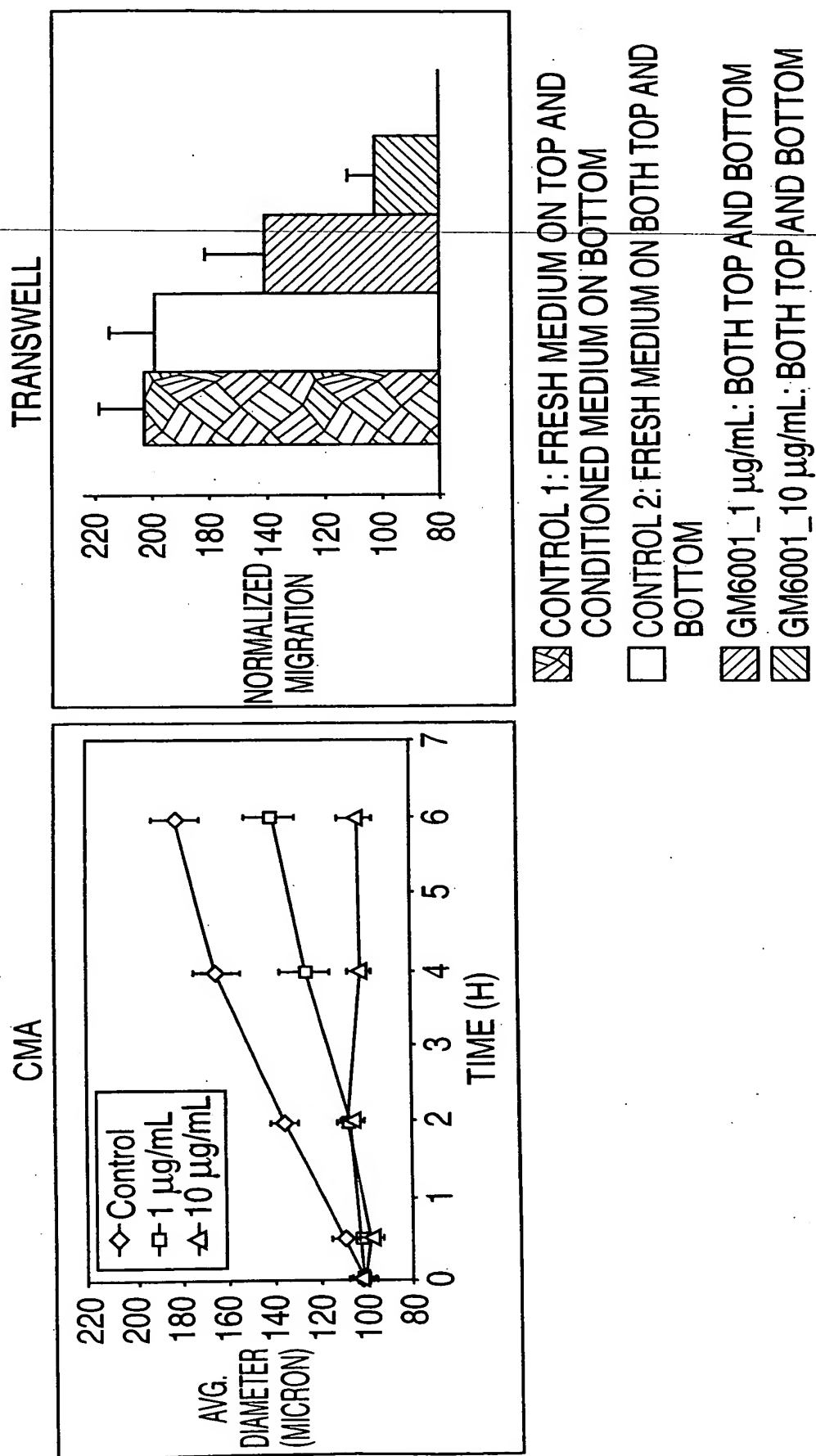
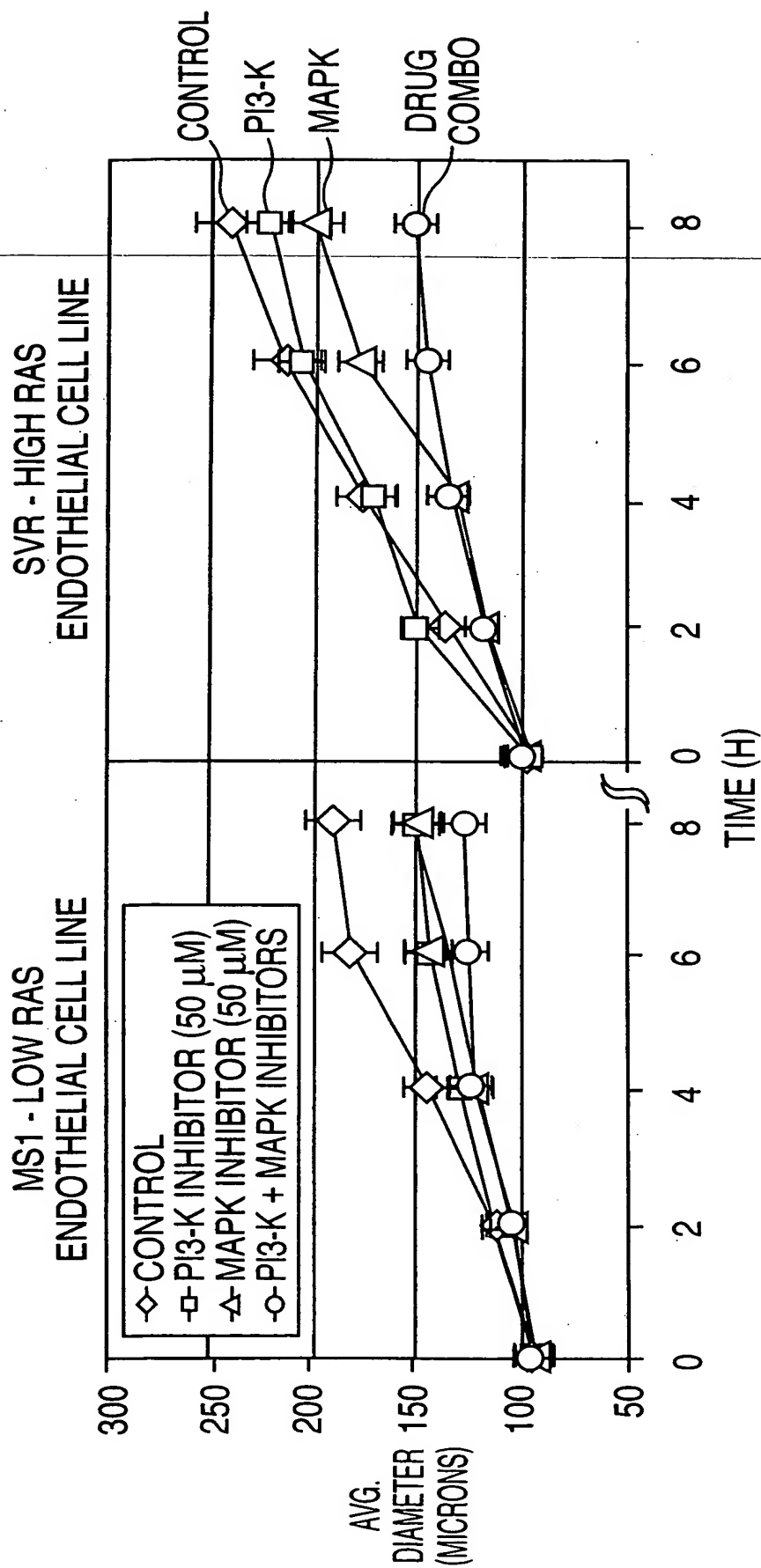


FIG. 22



•INHIBITION OF PI3-KINASE CAUSED A STRONGER INHIBITORY EFFECT IN MS1 (LOW RAS)

•INHIBITION USING BOTH PI3-K (LY90049) AND MAPK (PD98059) GENERATED AN ADDITIVE SUPPRESSION OF MIGRATION

FIG. 23

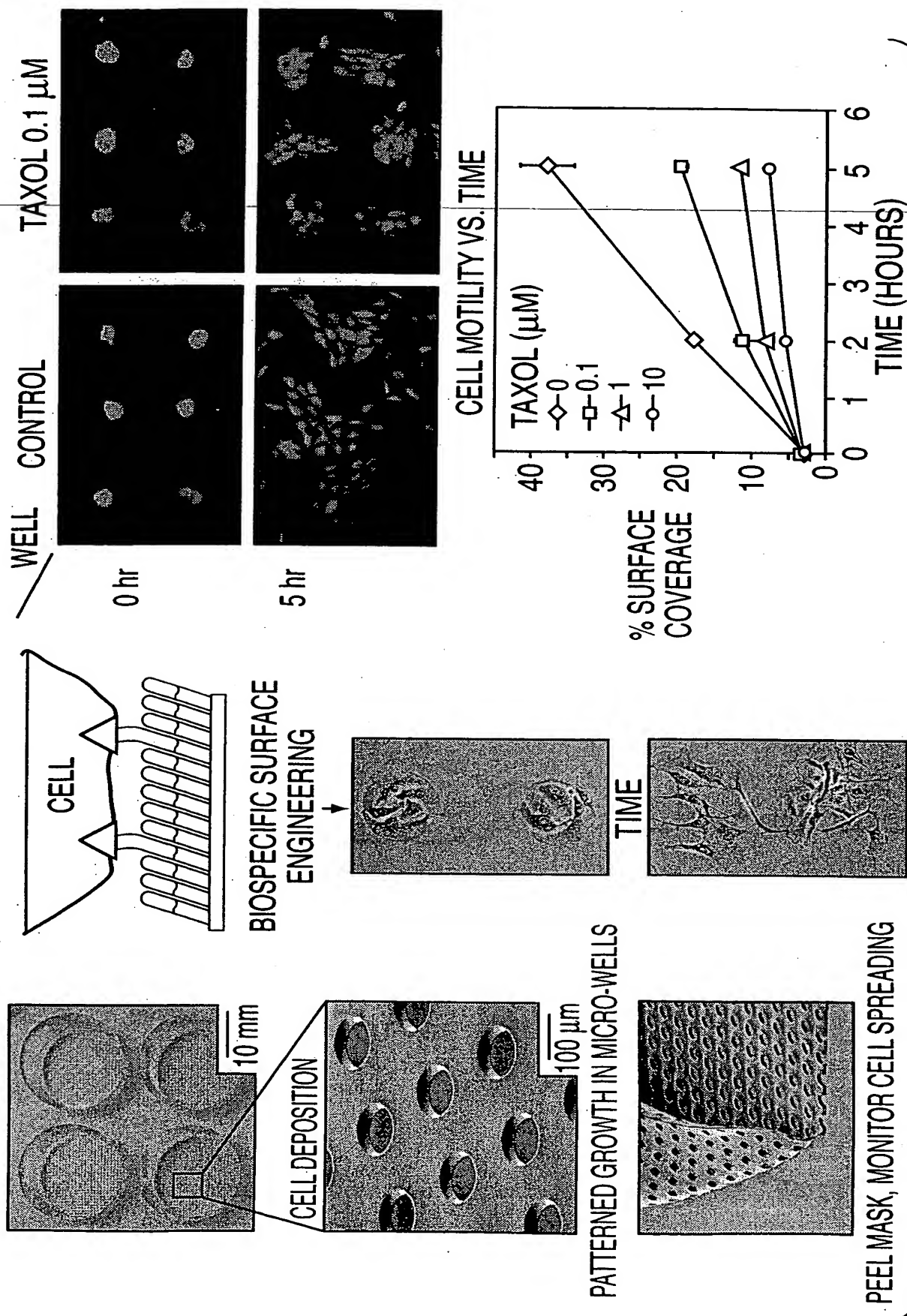


FIG. 24

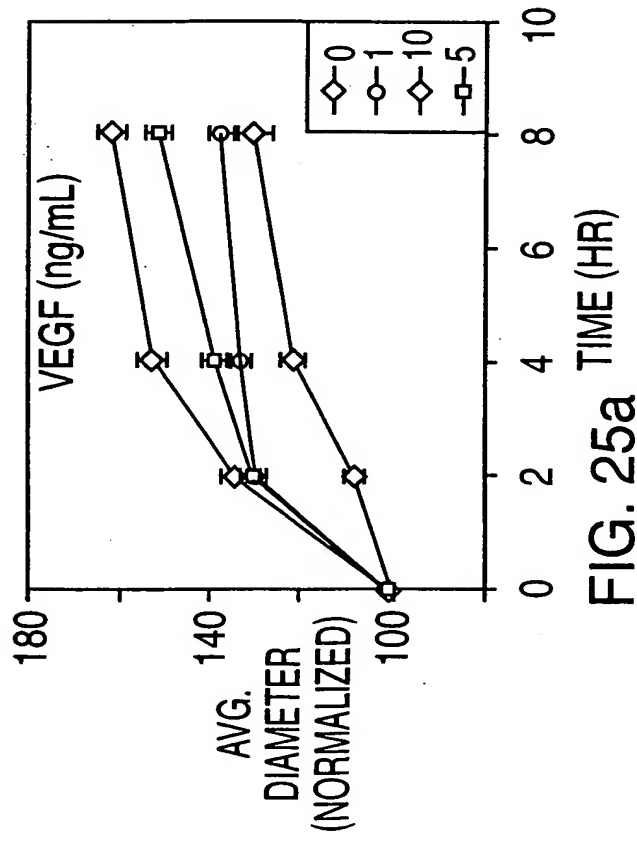


FIG. 25a

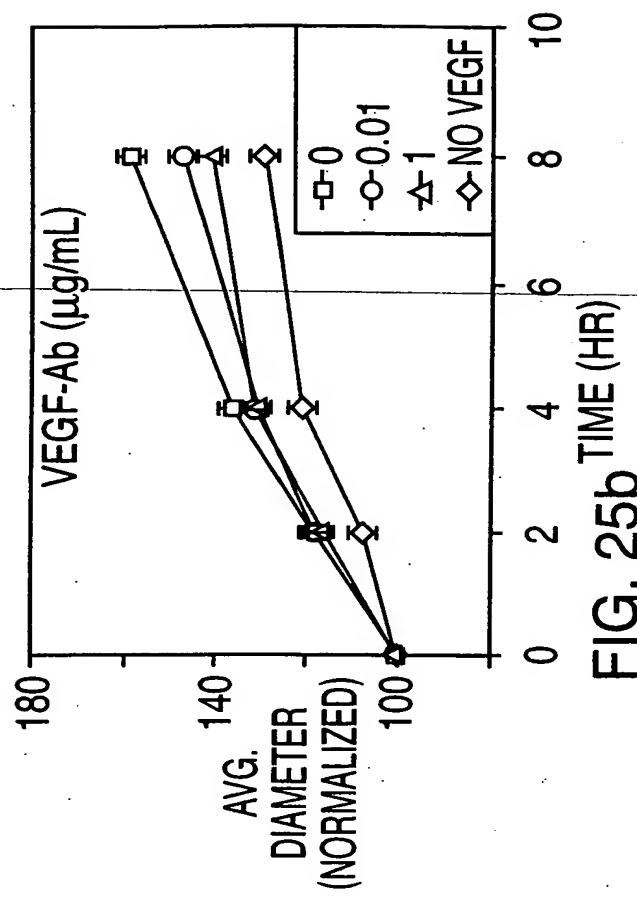


FIG. 25b

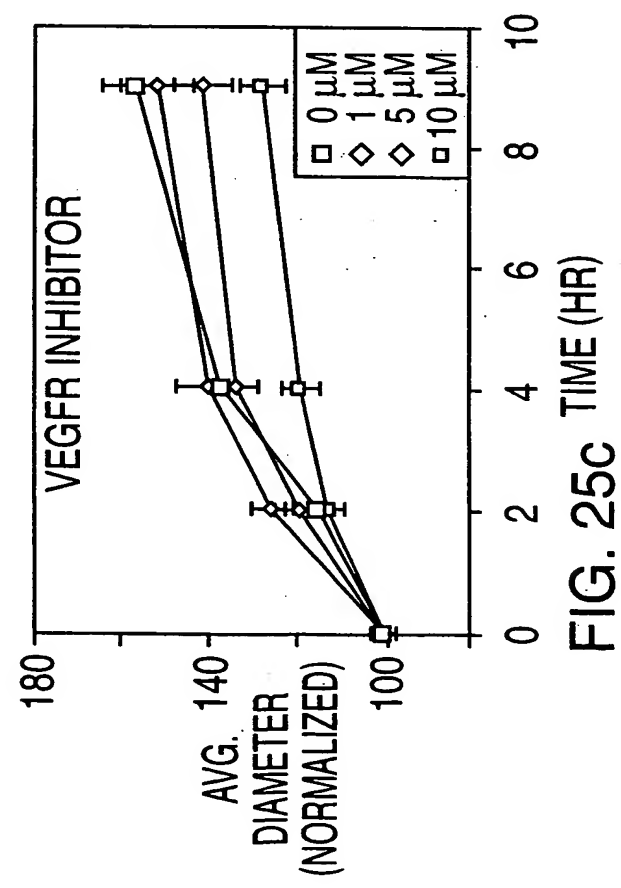


FIG. 25c

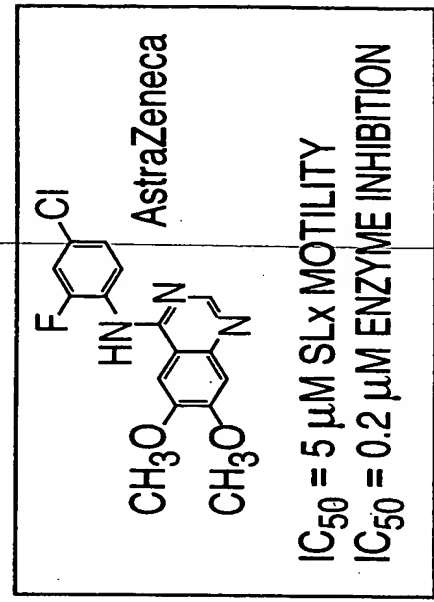
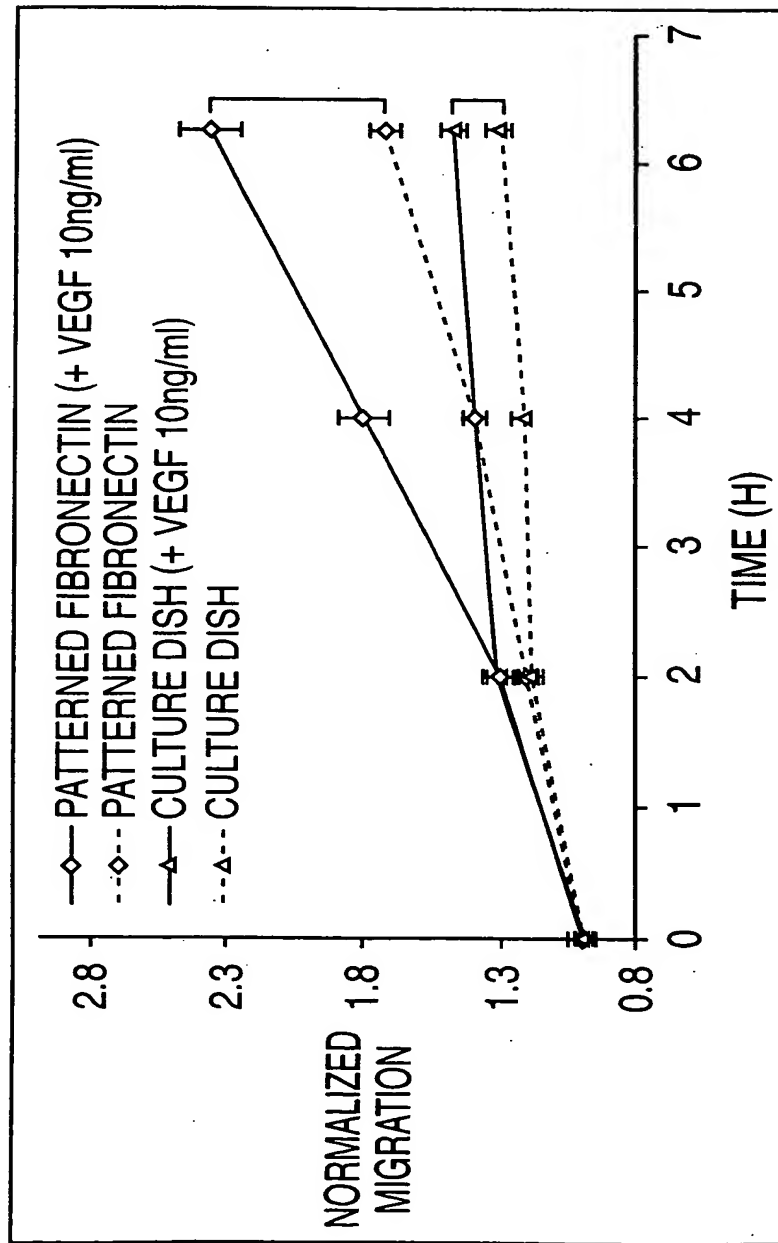
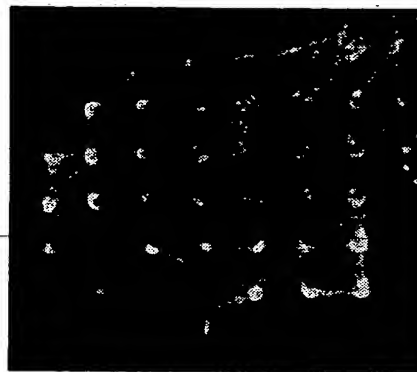


FIG. 25d

PATTERNED FIBRONECTIN VS TC PLASTIC



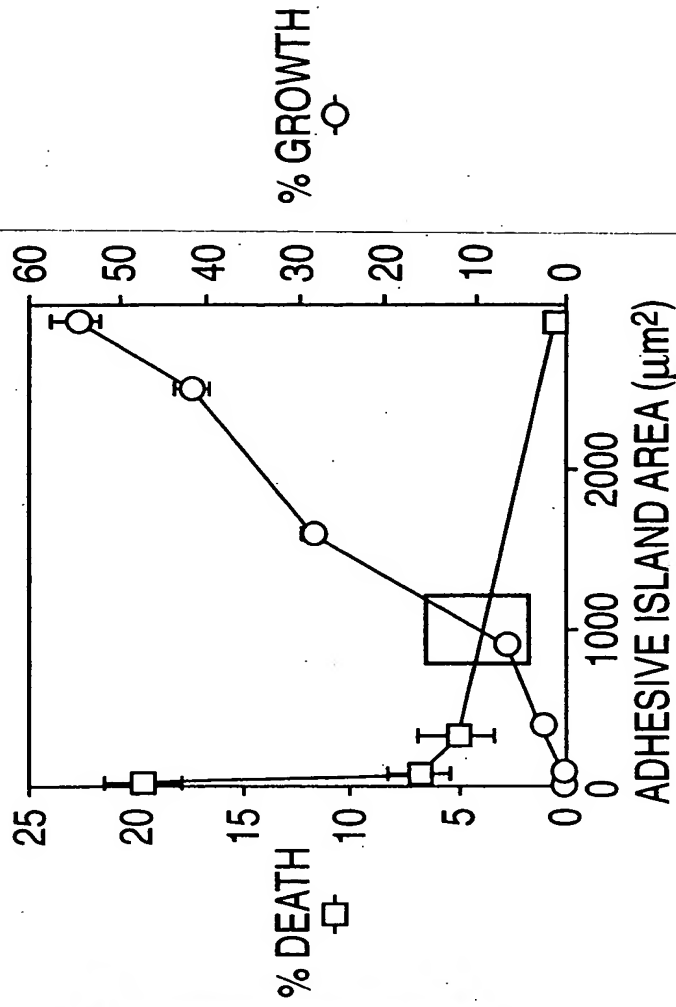
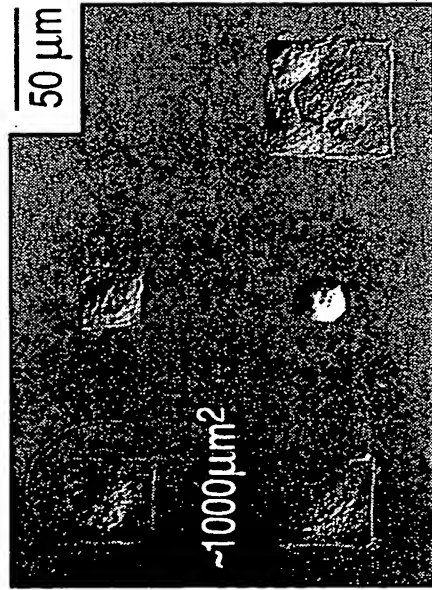
SURFACE - SPECIFIC CELLULAR
RESPONSE USING FOCAL
ADHESION MICROPATTERNING



— ACTIN
— VINCULIN

SURFACE CHEMISTRY AND MICROCONTACT PRINTING LEAD
TO MORE ROBUST MOTILITY MEASUREMENTS

FIG. 26



- ATTACHMENT AREA DETERMINES PHYSIOLOGICAL STATE
- PREPARATION OF HOMOGENOUS POPULATIONS OF CELLS

- CELL CONTROL FROM $1000 \mu\text{m}^2$
- CELL CYCLE ENTRY-GROWTH
- ONSET OF APOPTOTIC CASCADE
- SWITCH TO DIFFERENTIATION

FIG. 27

TIME-DEPENDENT SWITCH FROM GROWTH TO DIFFERENTIATION

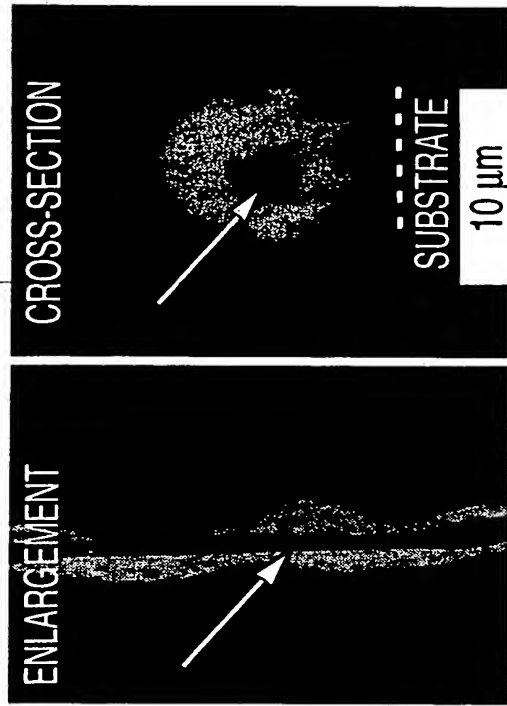
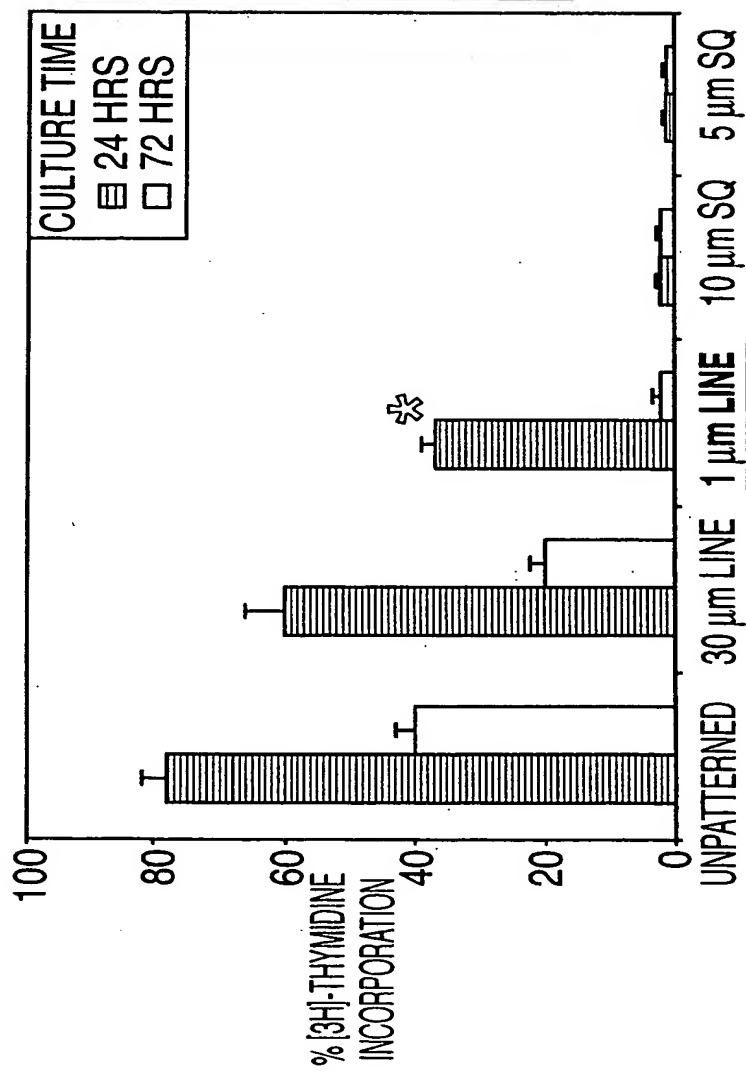
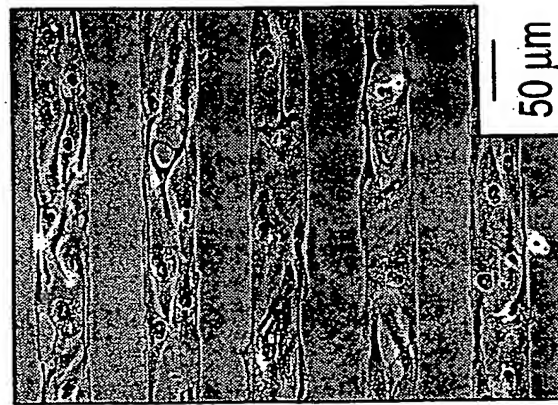


FIG. 28



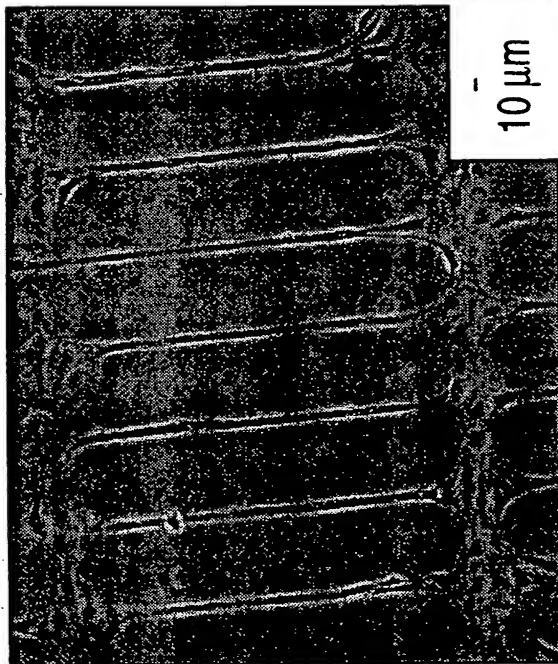
- ALIGNMENT
- POLARIZATION
- GROWTH



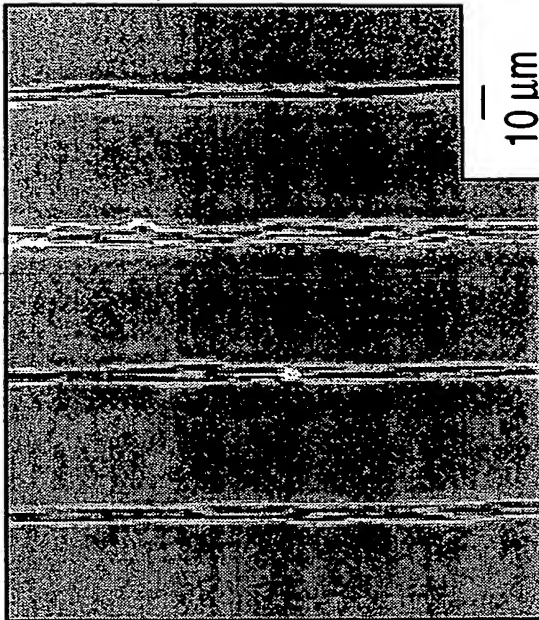
- ALIGNMENT
- POLARIZATION
- SIZE REDUCTION
- ARRAY



- ALIGNMENT
- POLARIZATION
- DIFFERENTIATION ARRAY



10 μm



10 μm

FIG. 29

EVALUATION OF CYTOSKELETAL STABILITY AND REARRANGEMENT

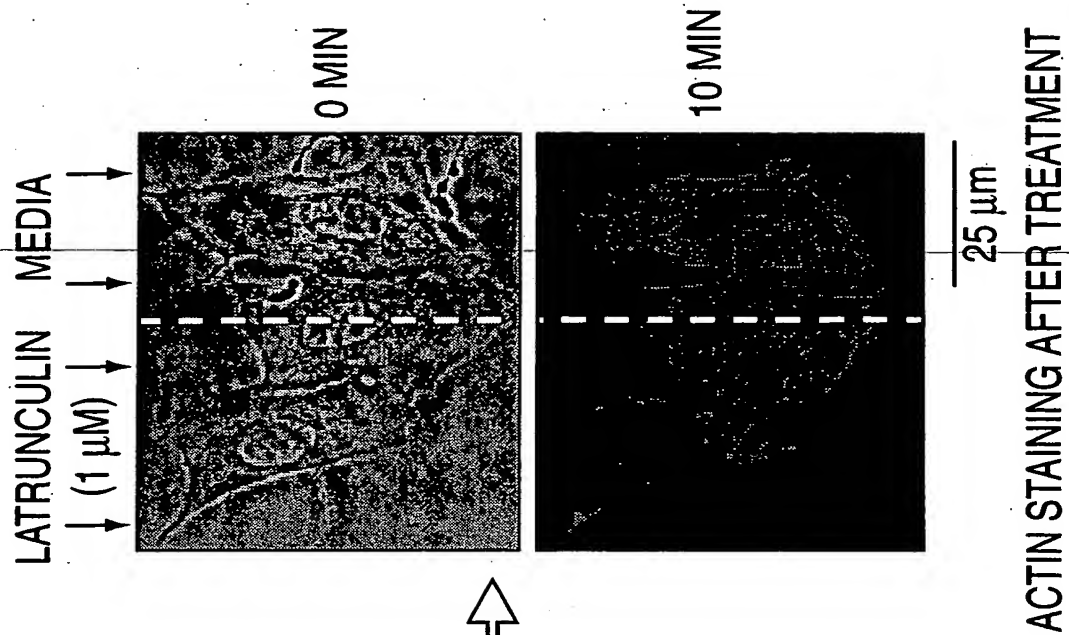
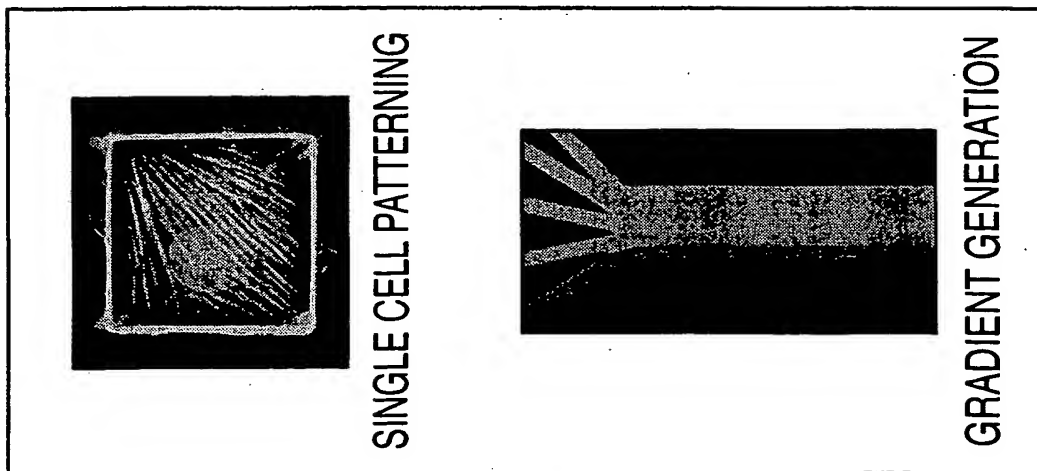


FIG. 30

1100

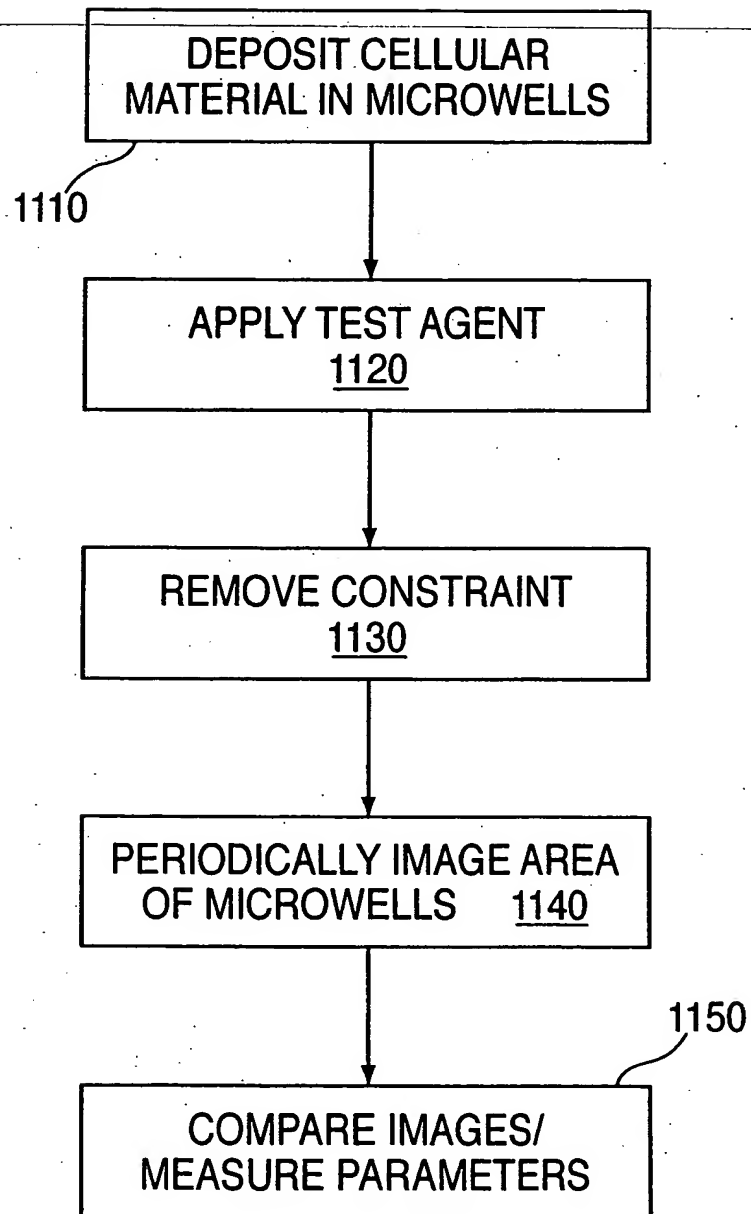


FIG. 31

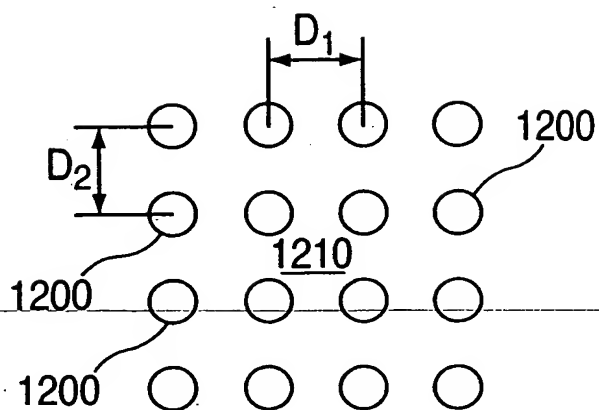


FIG. 32

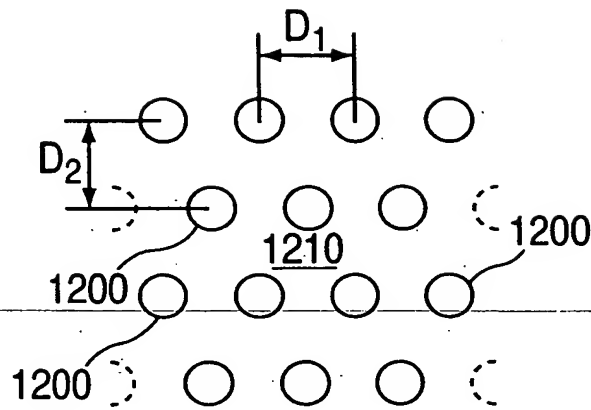


FIG. 33

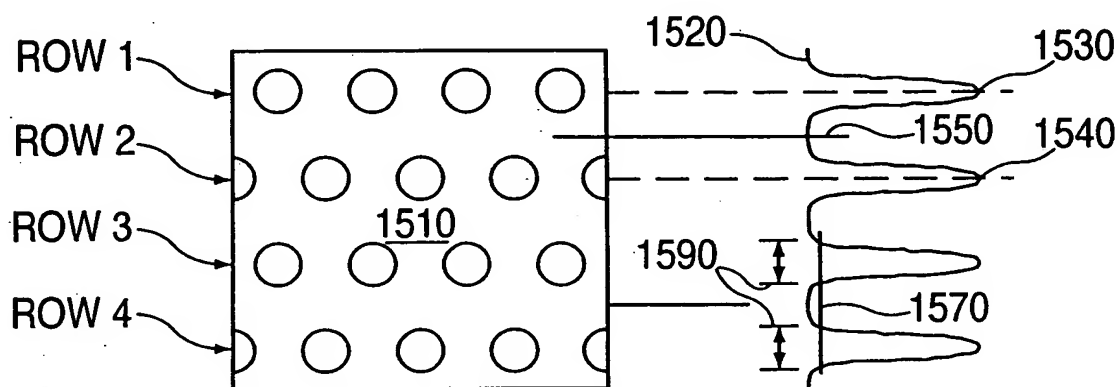


FIG. 35

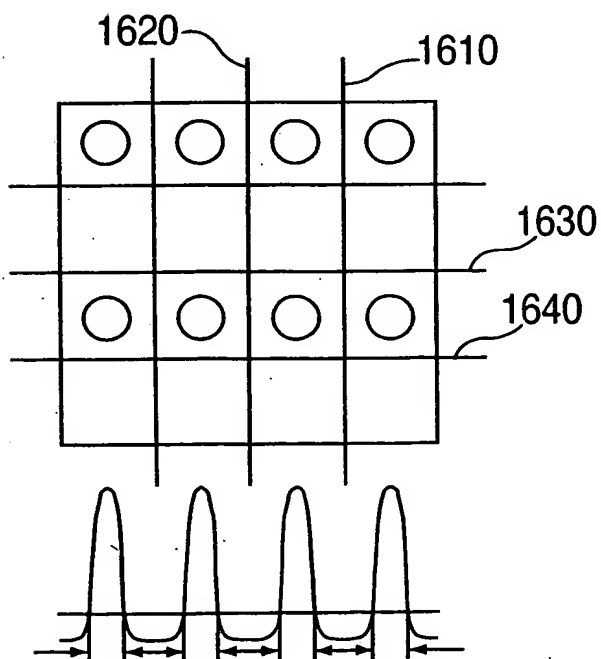


FIG. 36

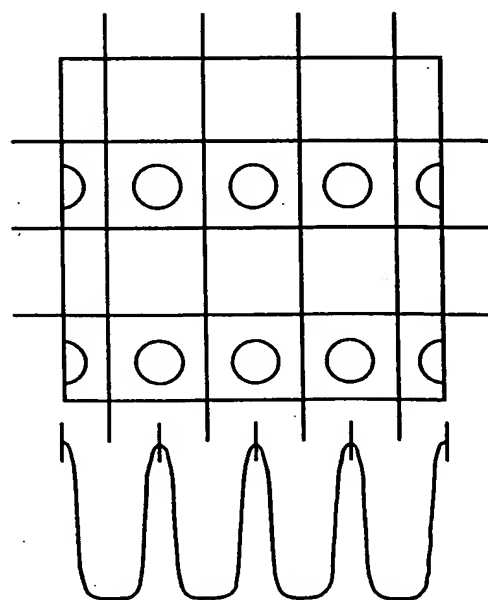


FIG. 37

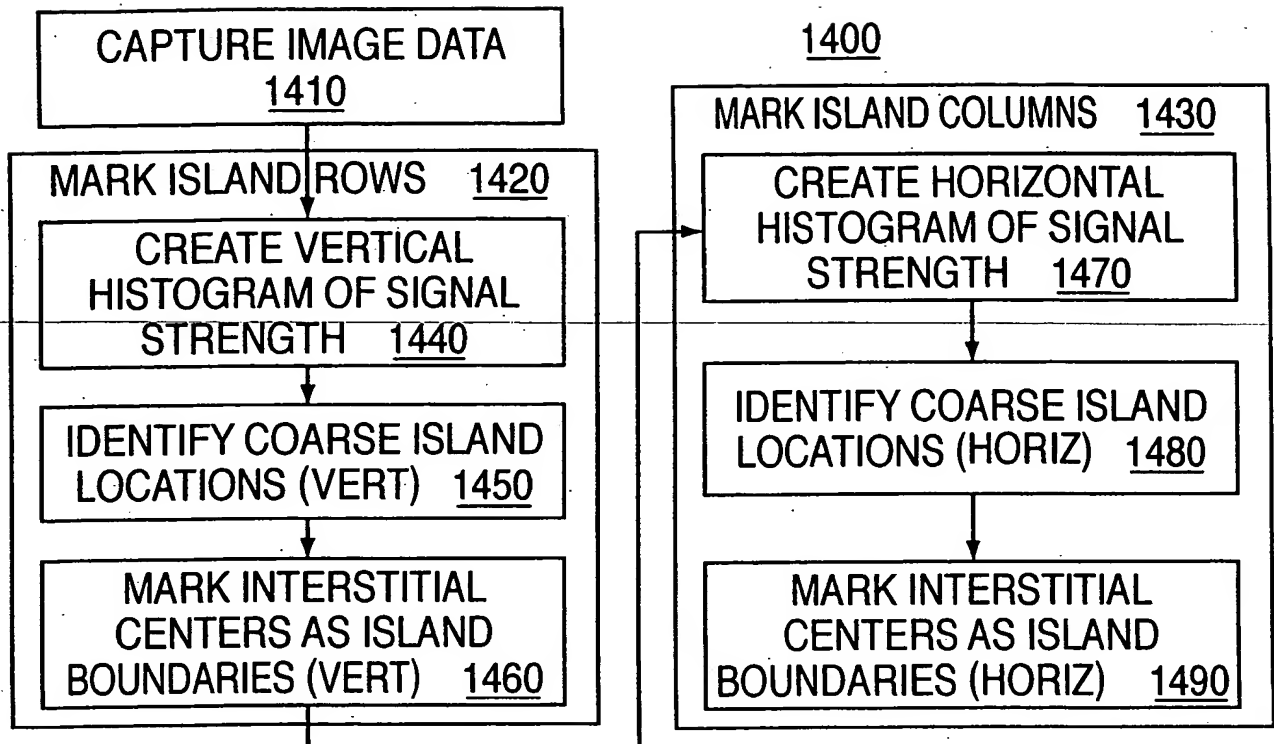


FIG. 34

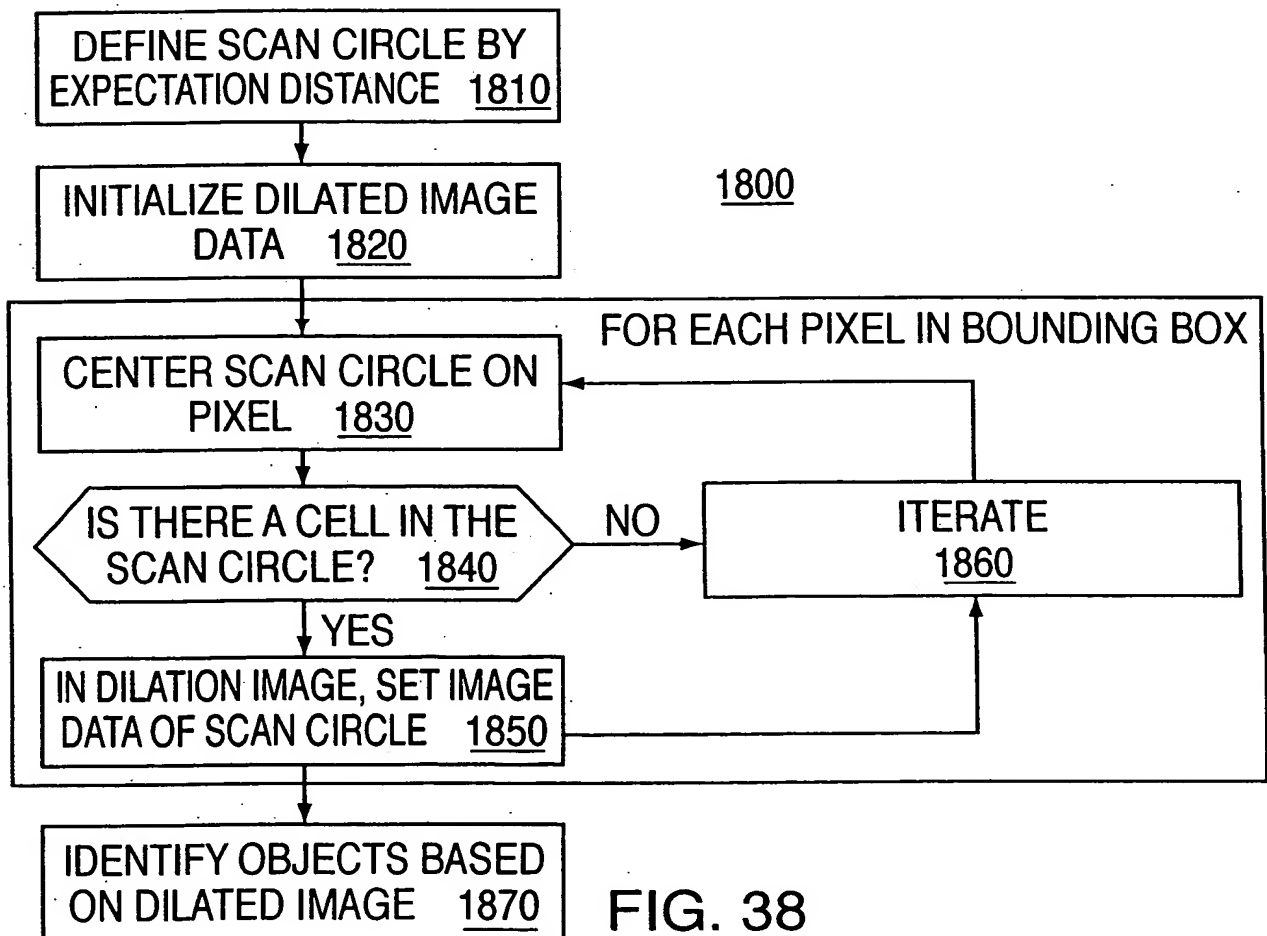
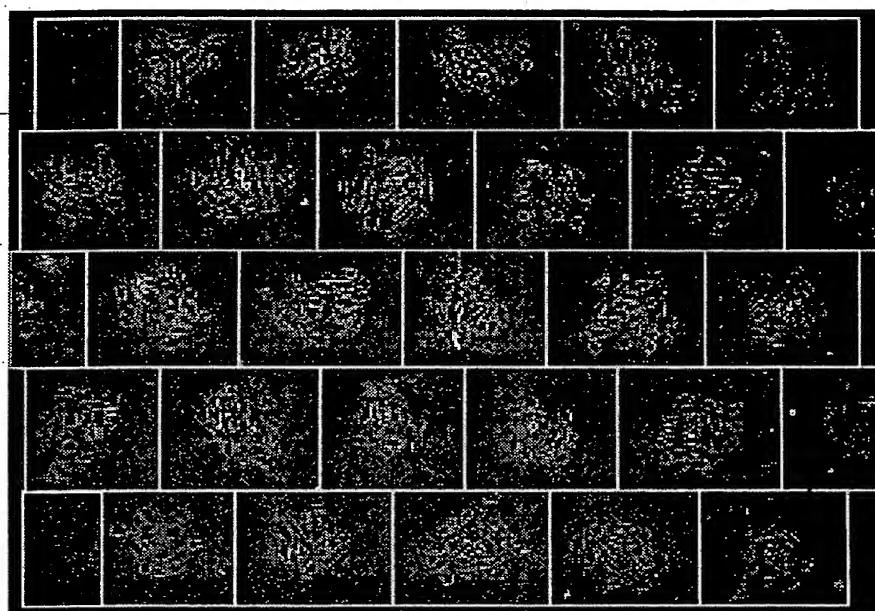


FIG. 38



BOX
BOUNDARIES

FIG. 39
ISLANDS

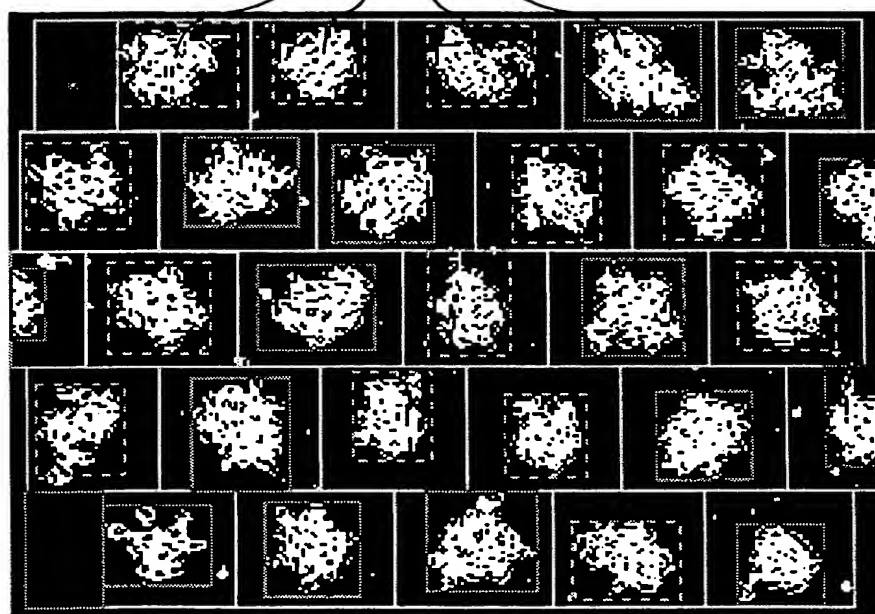
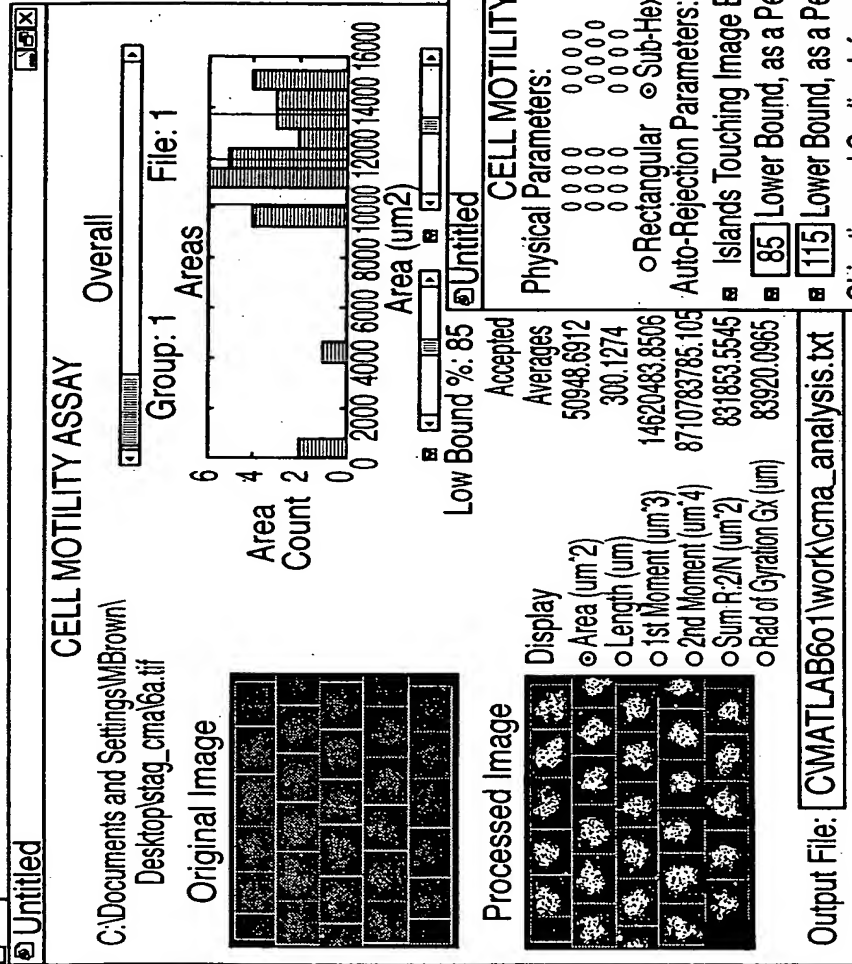


FIG. 40

☒ Untitled
CELL MOTILITY ASSAY
 Current File list for Group: 1 Currently Displayed Group

☒ C:\Documents and Settings\MBrown\Desktop\stag_cma16a.tif
☐ Add File to current Group
☐ Clear Current Group
☐ Options
☒ Begin Processing

Processing Group 1 images C:\Documents and Settings\MBrown\Desktop\stag_cma16a.tif
 Output File:
 [C:\MATLAB6p1\work\cma_analysis.txt]



CELL MOTILITY ASSAY OPTIONS

Physical Parameters:

Approximated Cell Diameter (microns)
 Threshold Factor
 Auto-Rejection Parameters:
☐ Rectangular ☒ Sub-Hex

Islands Touching Image Boundary

☒ [85] Lower Bound, as a Percentage of the Median Area
☒ [115] Lower Bound, as a Percentage of the Median Area

Objective and Scaling Information:

☐ Carl Zeiss 5X (~483 pixels/mm) ☐ Olympus 5X (~242 pixels/mm)
☐ Carl Zeiss 10X (~972 pixels/mm) ☐ Olympus 10X (~483 pixels/mm)
☐ Carl Zeiss 20X (~1964 pixels/mm) ☐ Olympus 20X (~964 pixels/mm)
☐ Carl Zeiss 40X (~3950 pixels/mm) ☐ Olympus 100X (~4900 pixels/mm)
☐ Carl Zeiss 63X (~6313 pixels/mm)
☐ Other [483] Pixels per Millimeter

Accept Cancel

FIG. 41

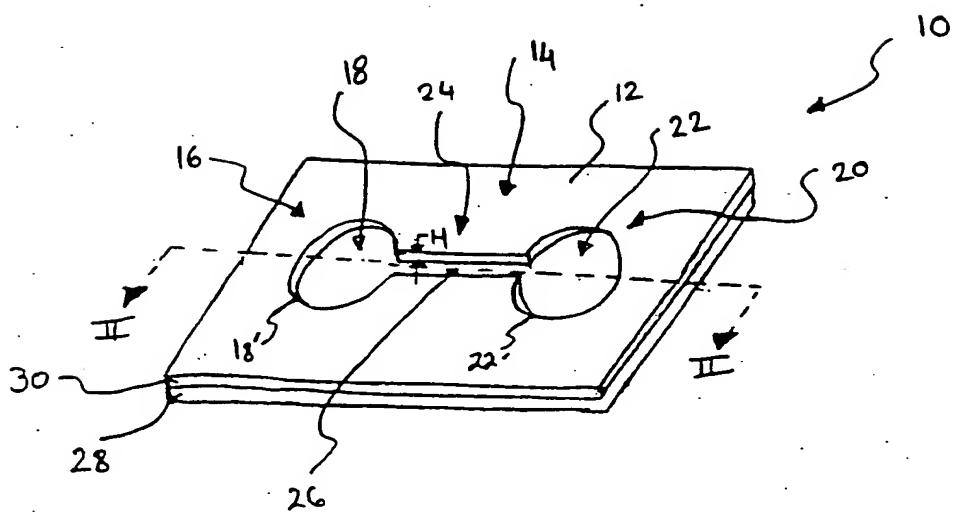


FIG. 42

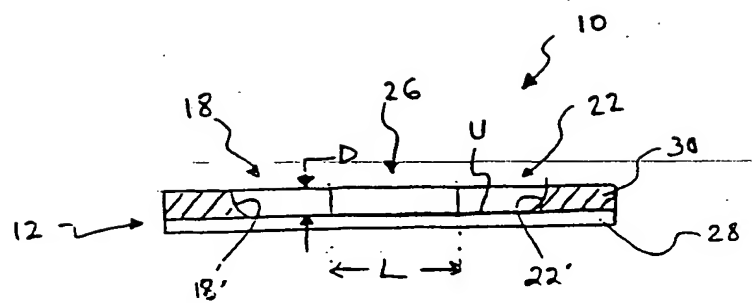


FIG. 43

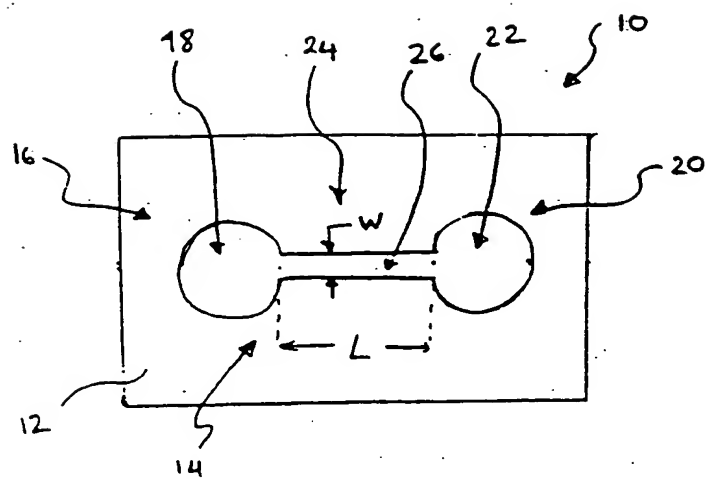


FIG. 44

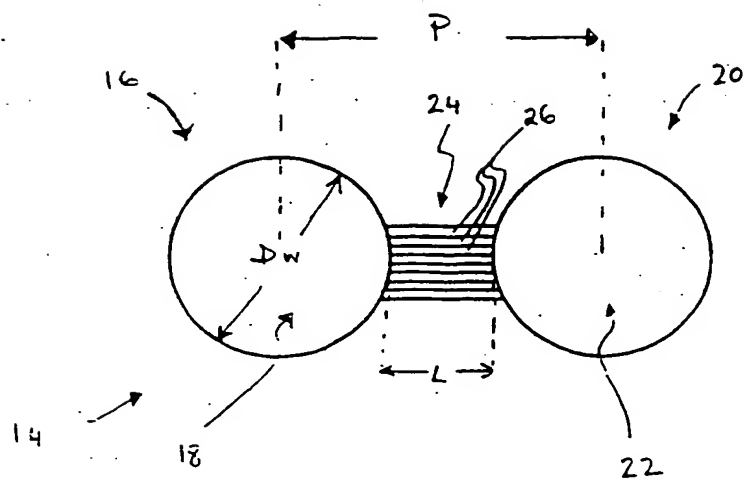


FIG. 45

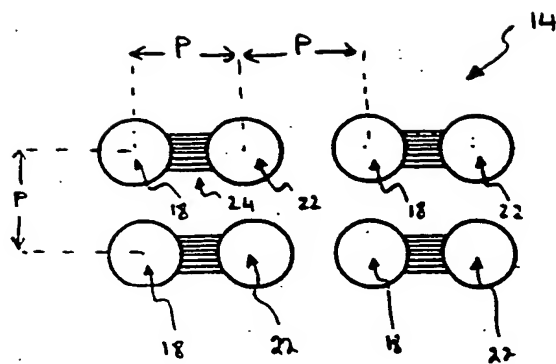


FIG. 46

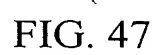


FIG. 47

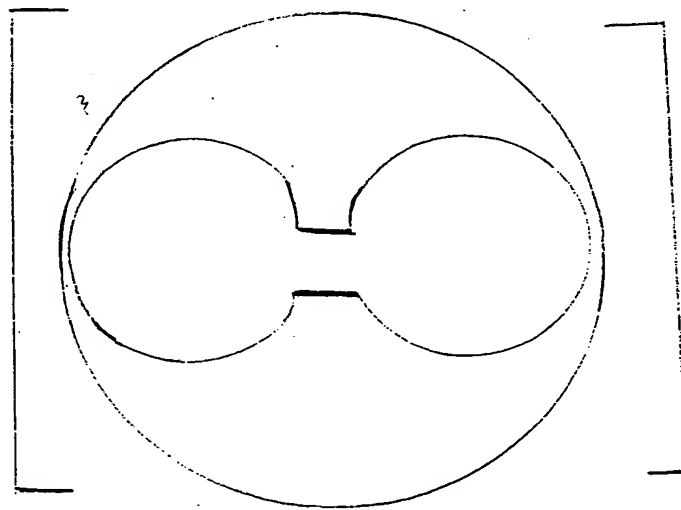
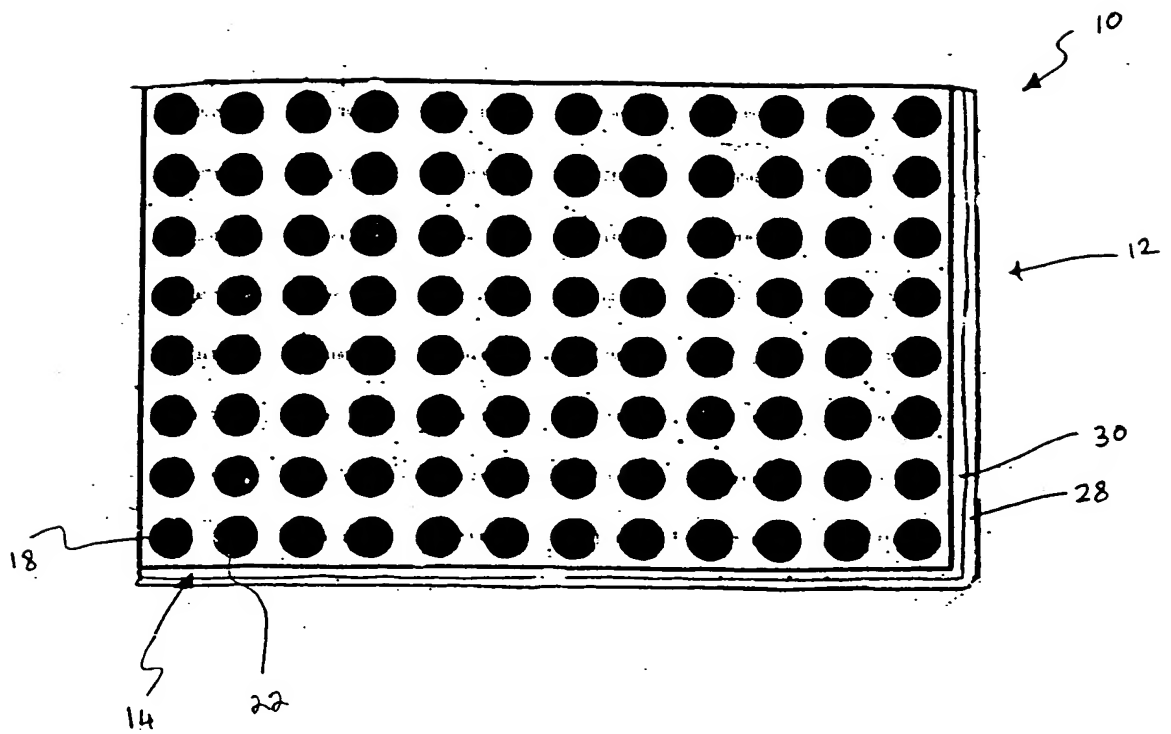


FIG. 47A

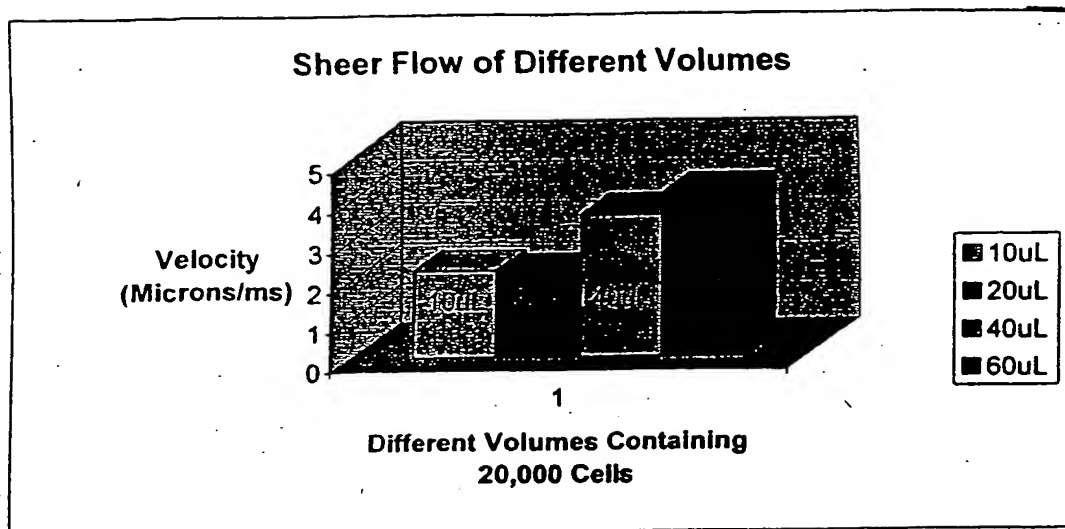


FIG. 48

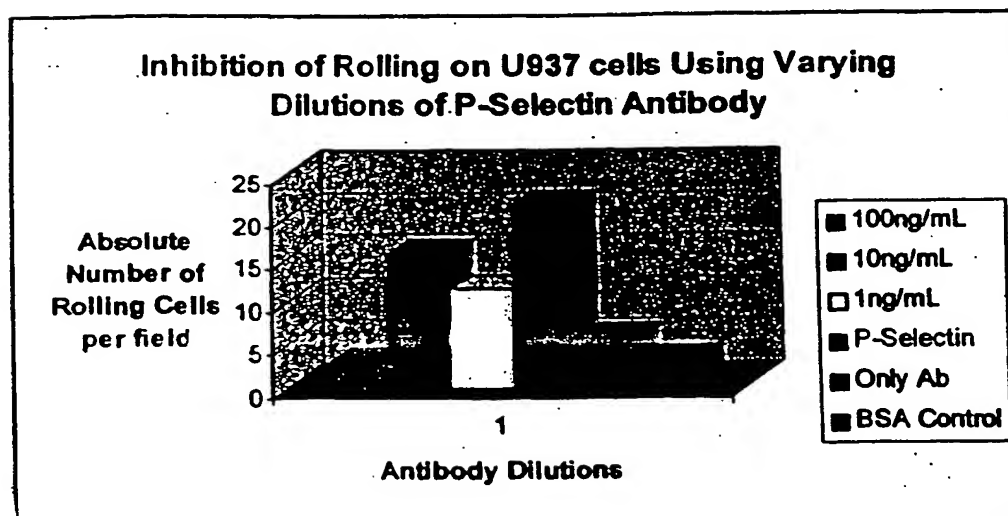


FIG. 49

Monocytic cell line (THP-1) rolling and adhering to P-selectin

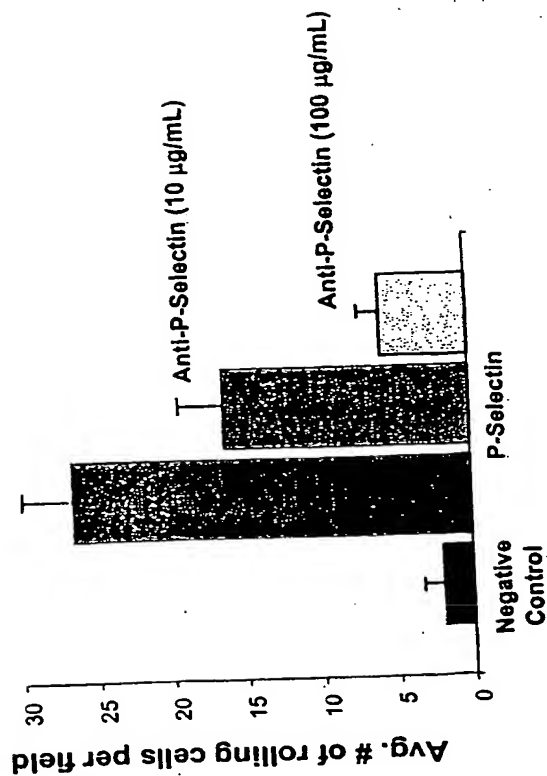
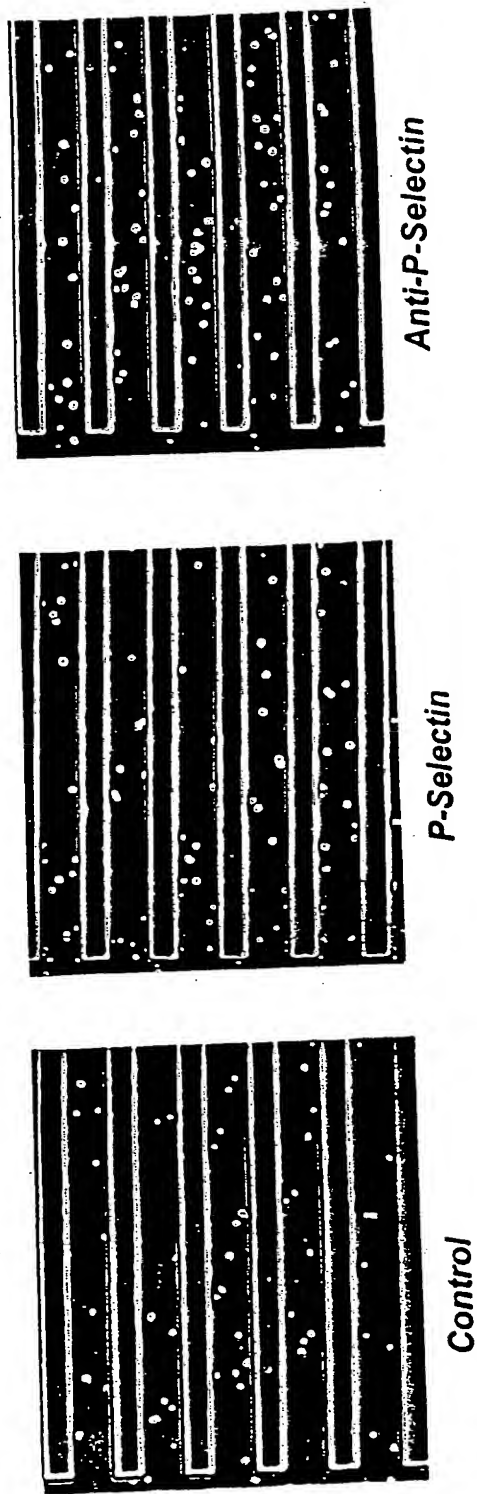


FIG. 50

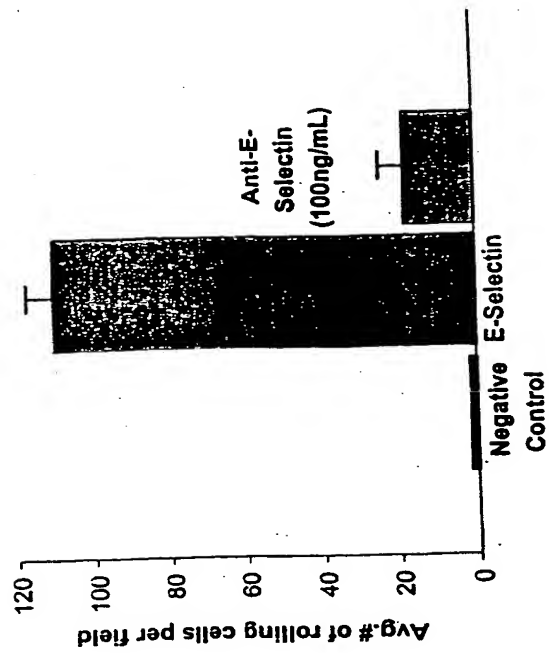
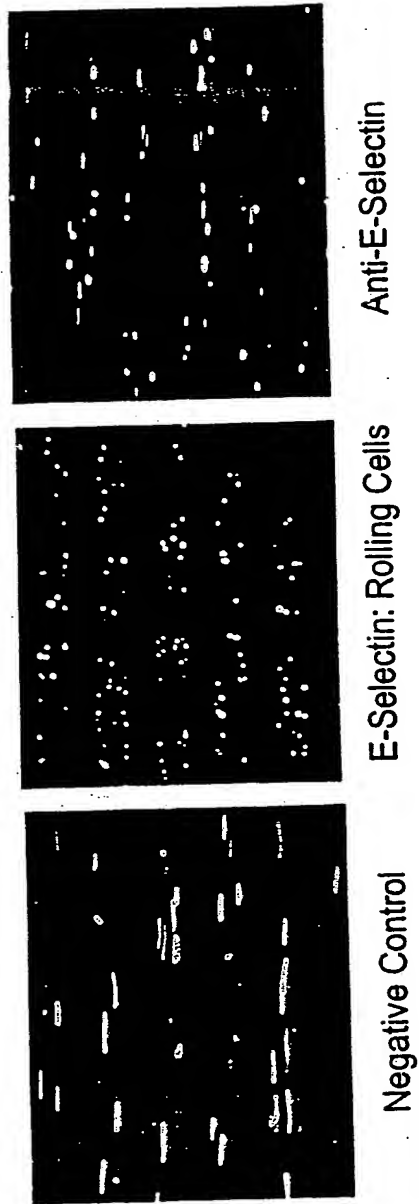


FIG. 51

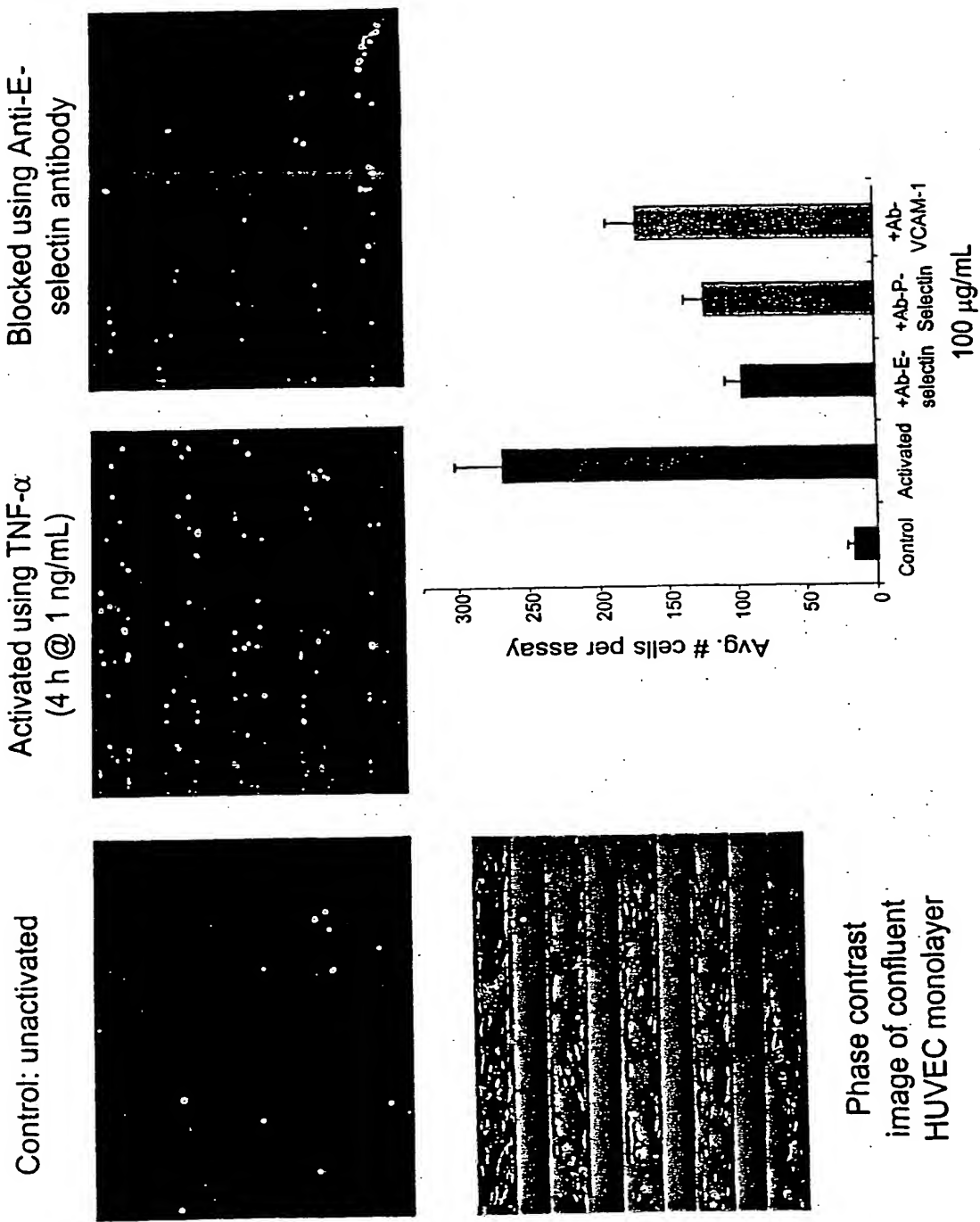


FIG. 52

Selective Activation of Endothelium

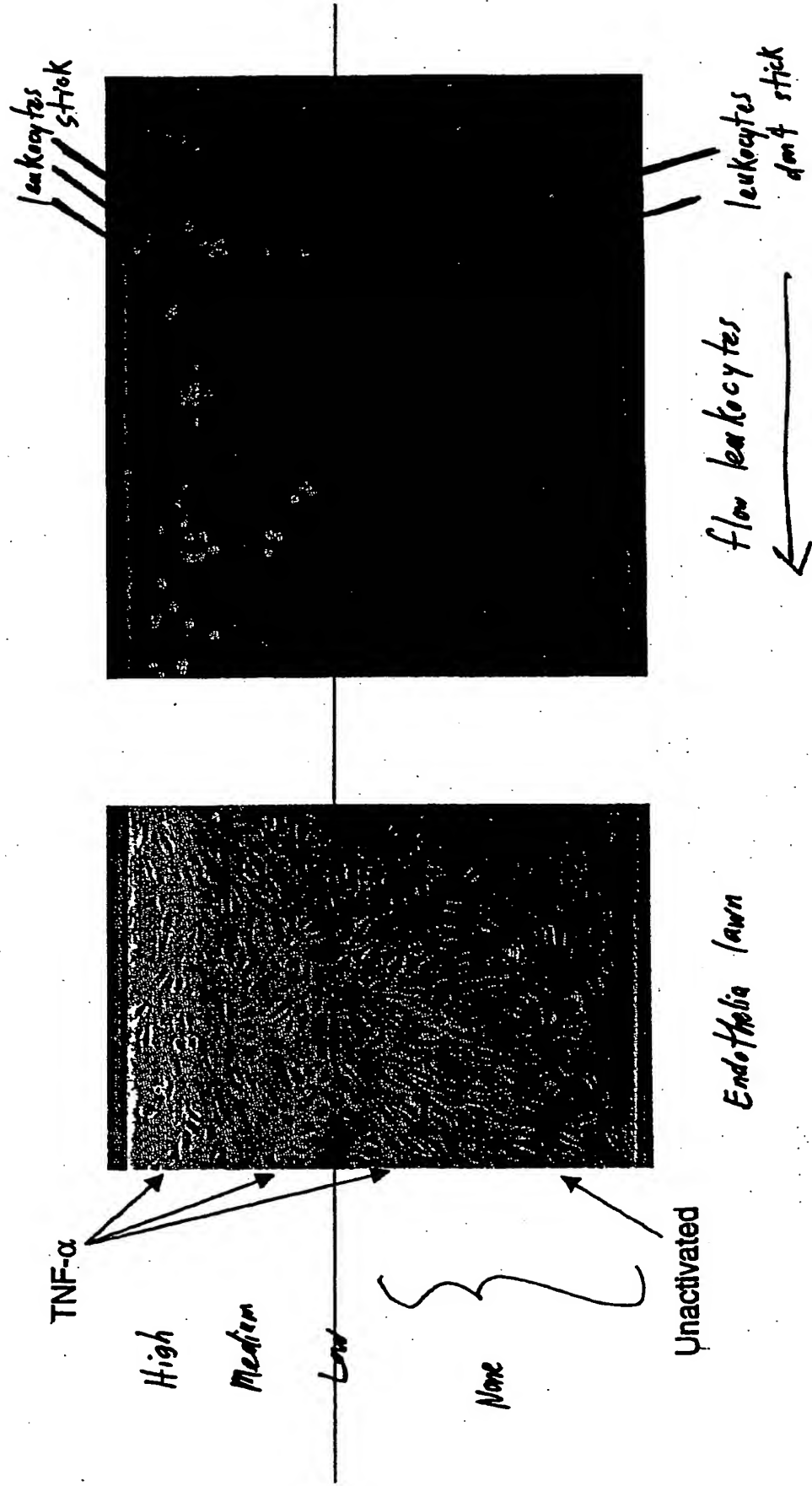


FIG. 53

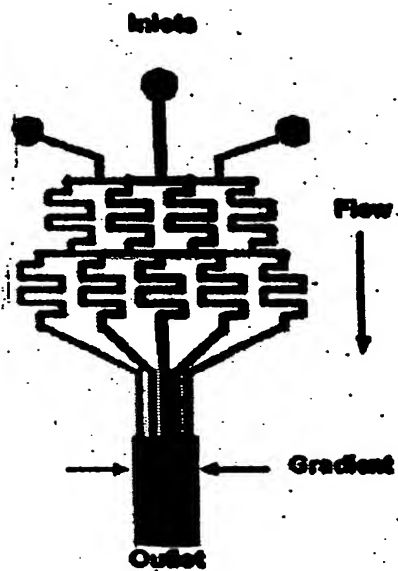


FIG. 54

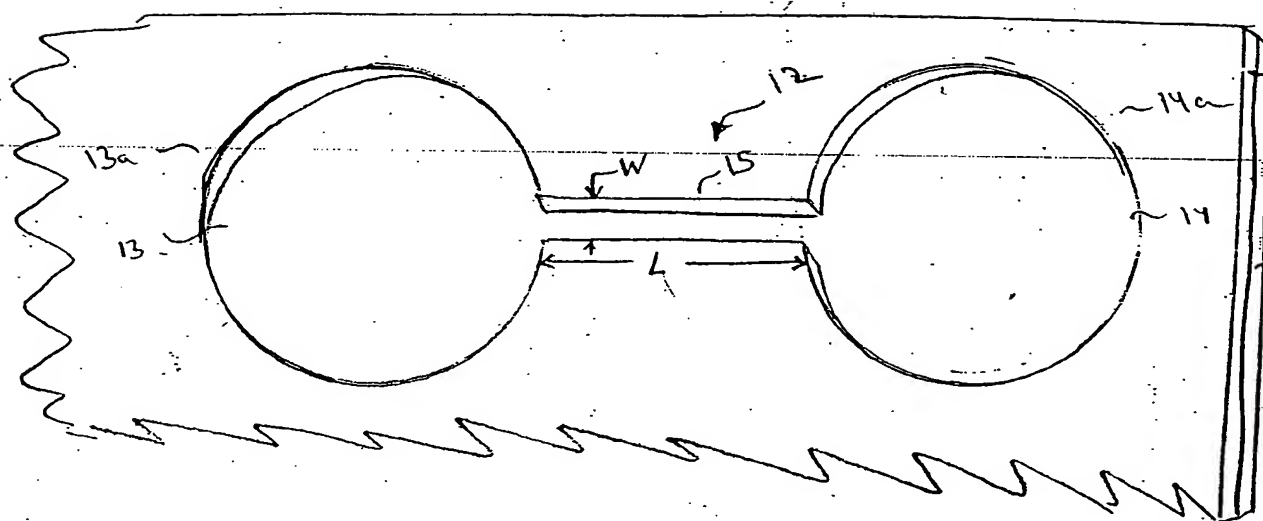


FIG. 55A

FIG. 55B

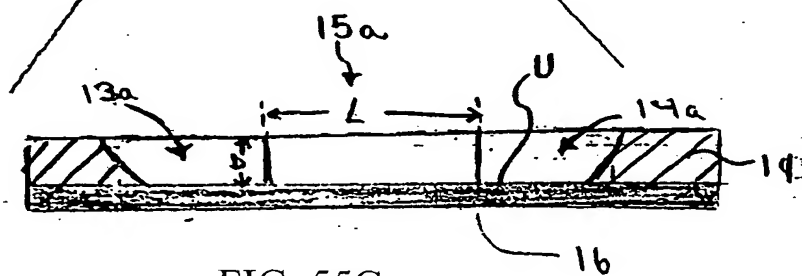
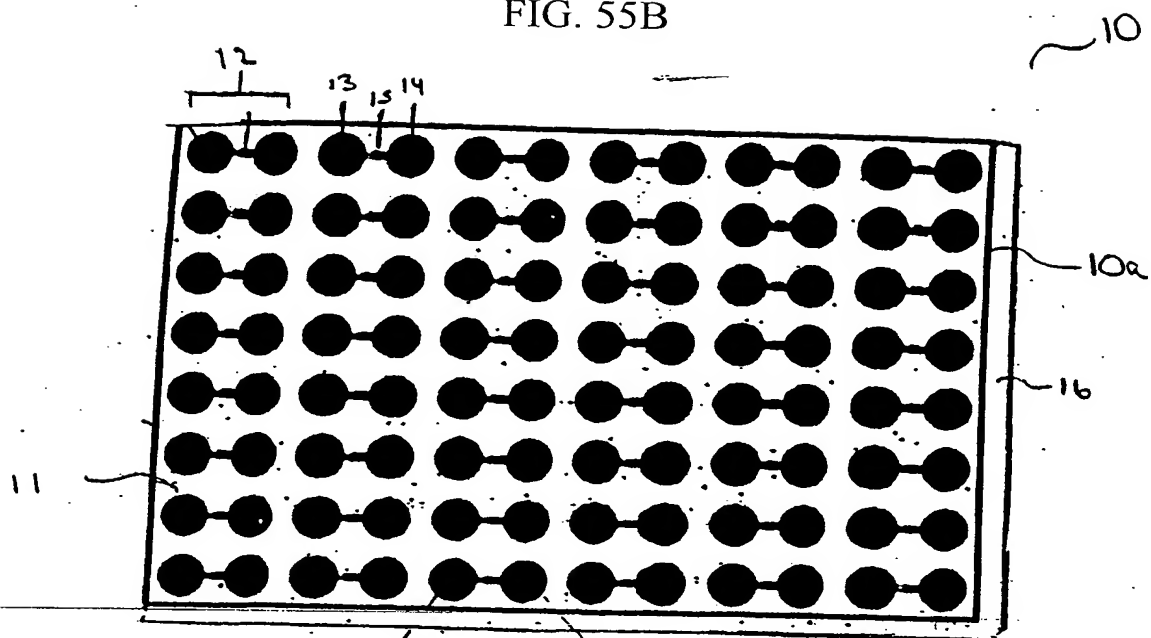
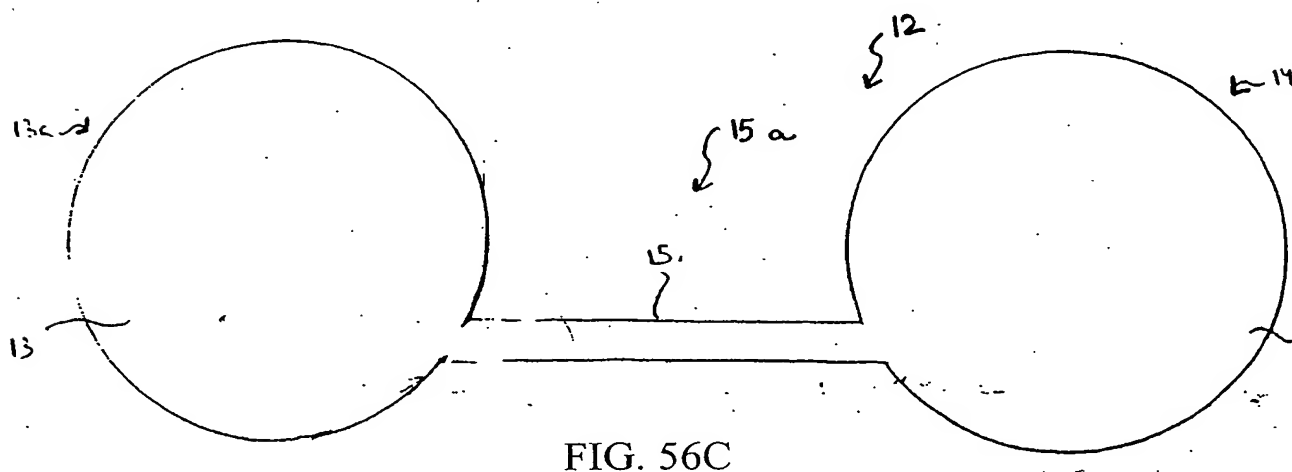
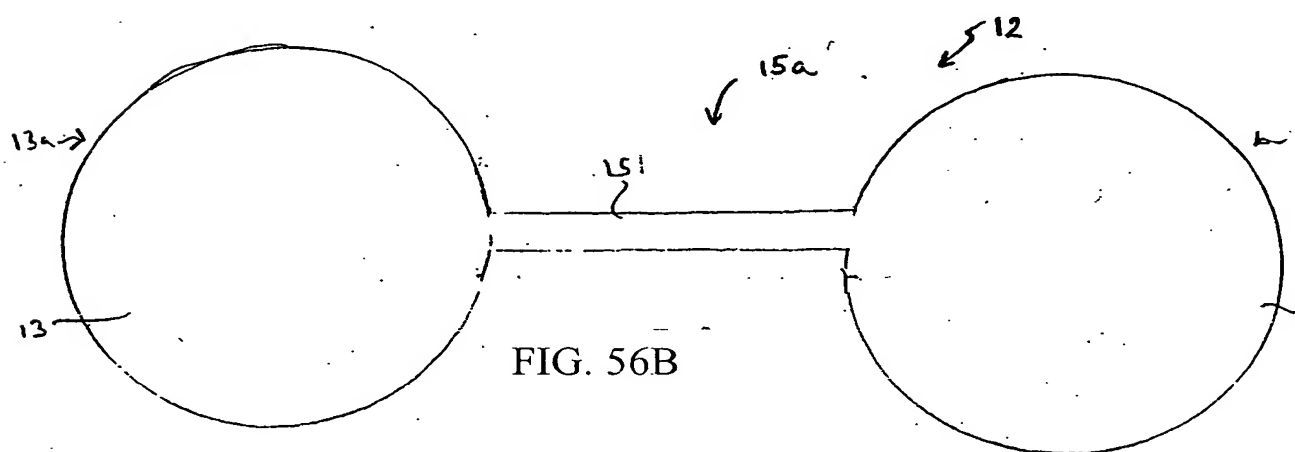
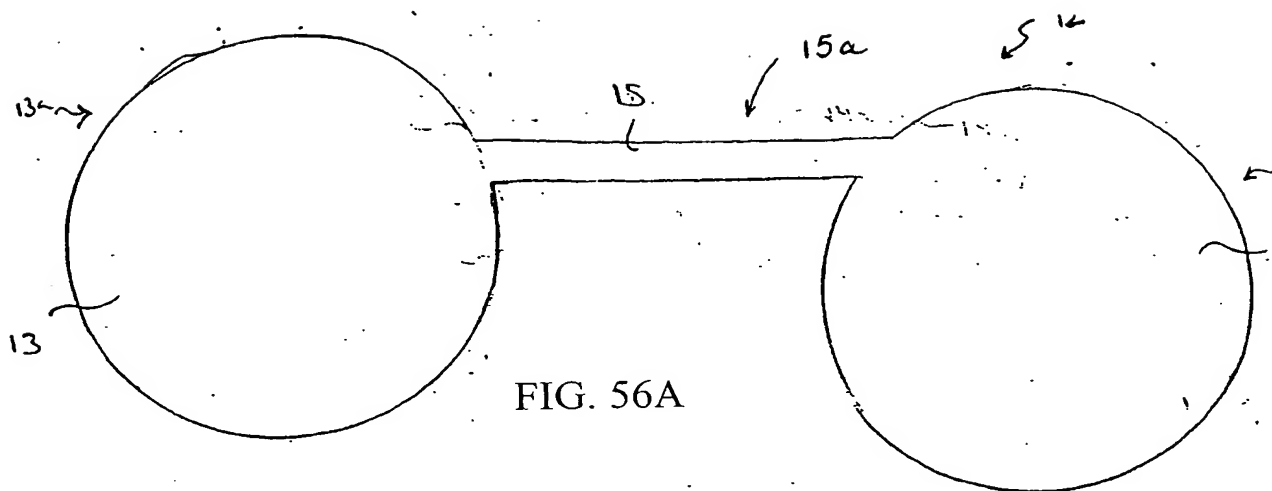


FIG. 55C



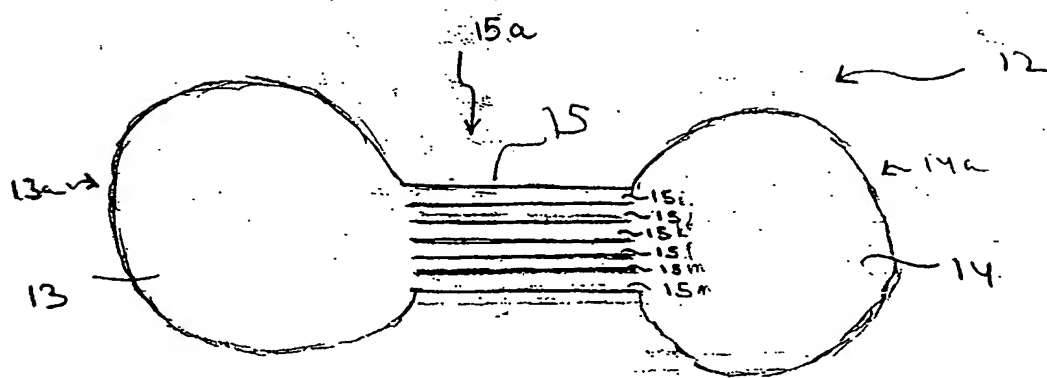


FIG. 57A

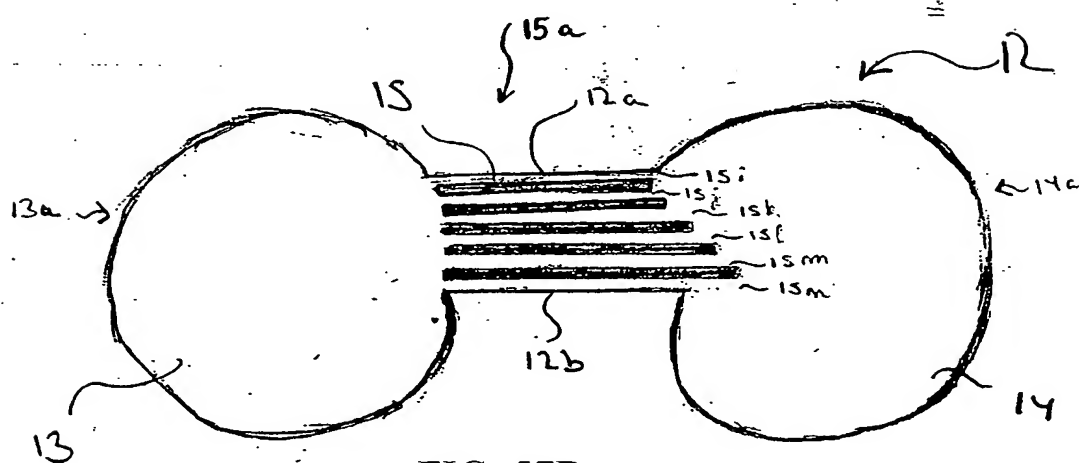


FIG. 57B

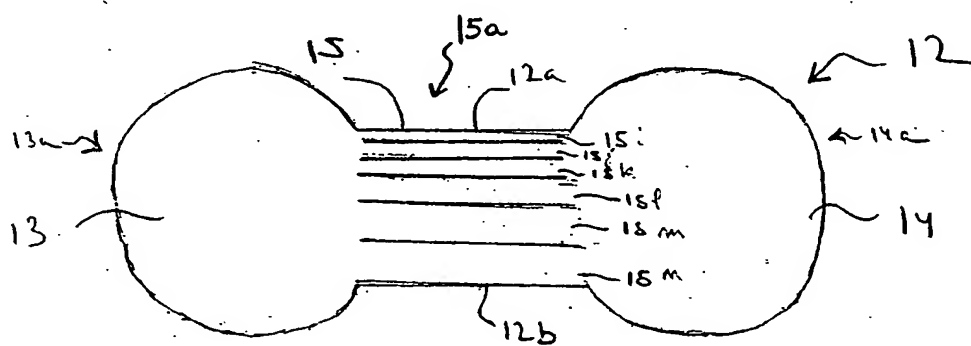


FIG. 57C

FIG. 58B

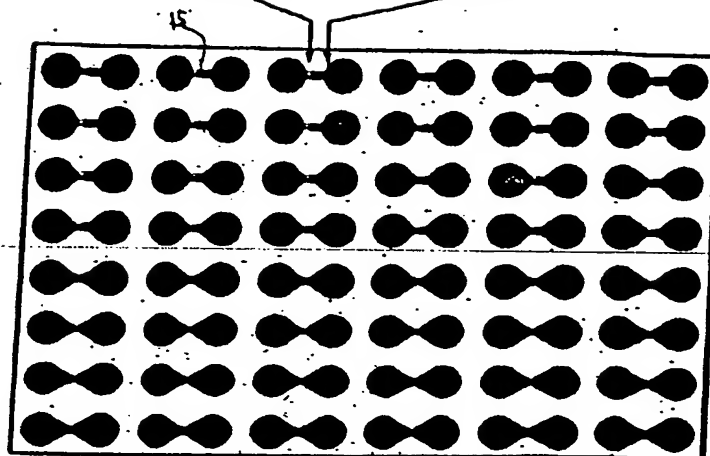
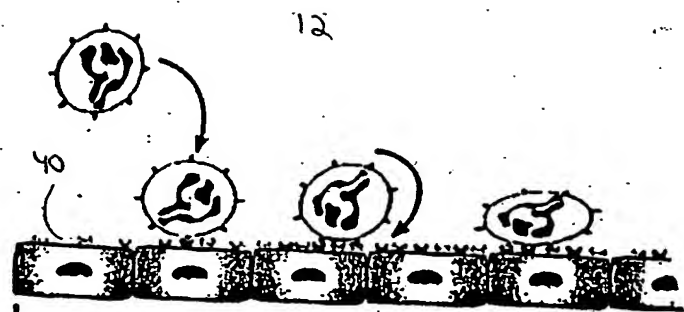


FIG. 58A

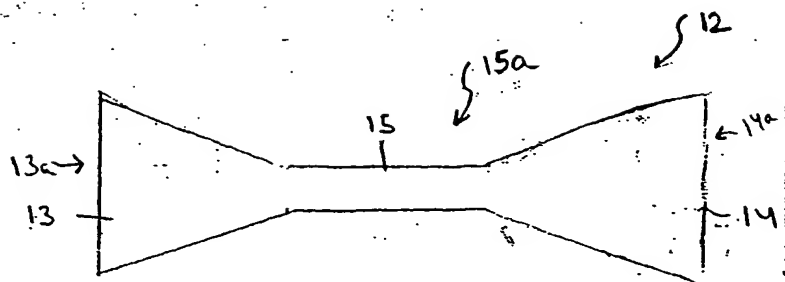


FIG. 59

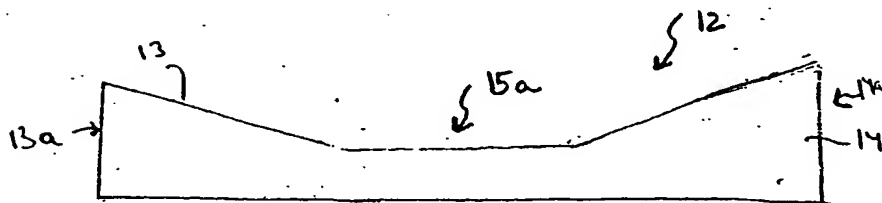


FIG. 60

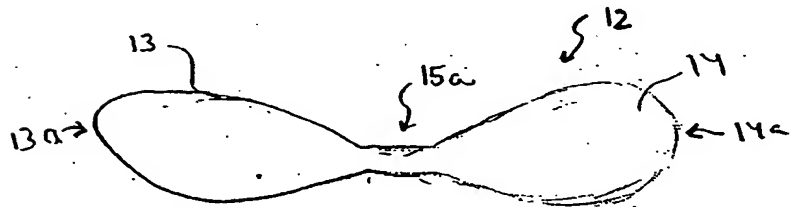


FIG. 61

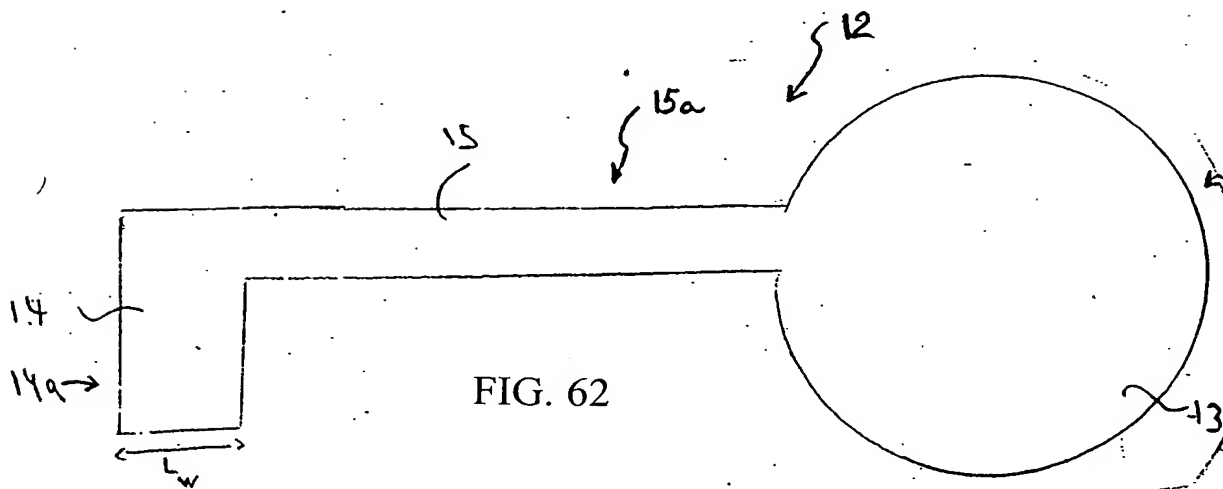


FIG. 62

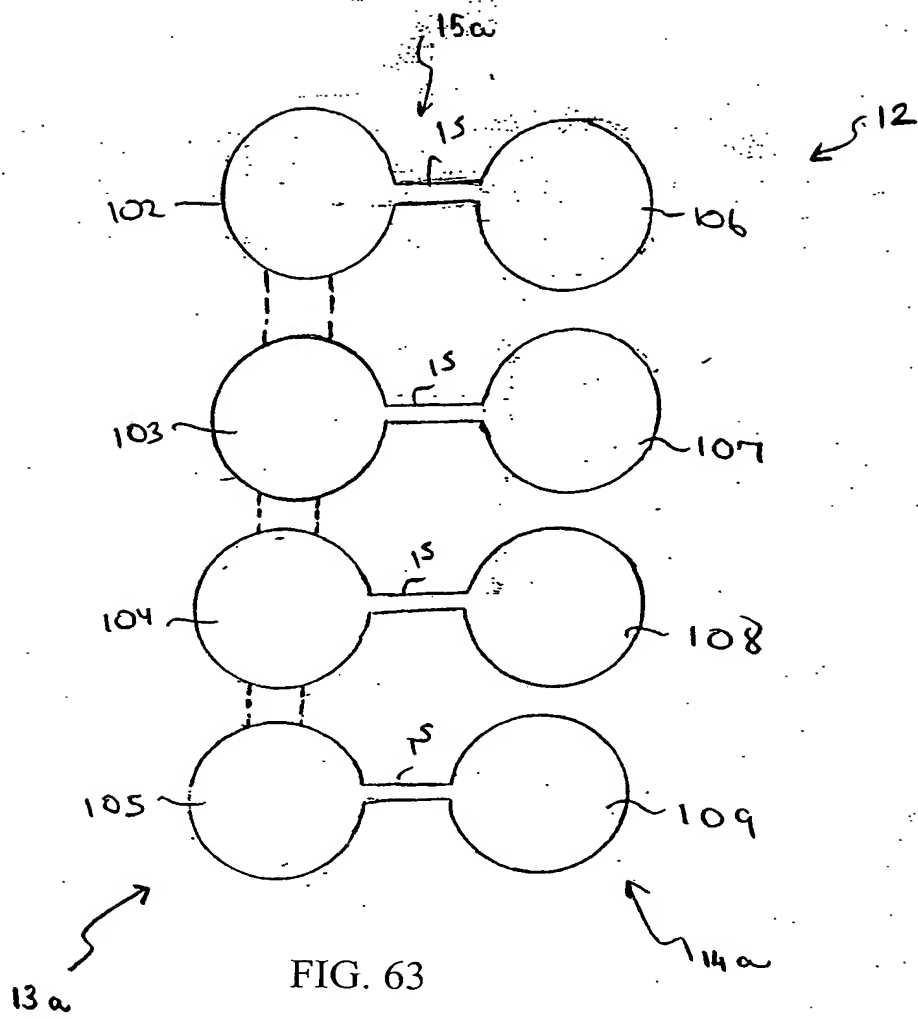


FIG. 63

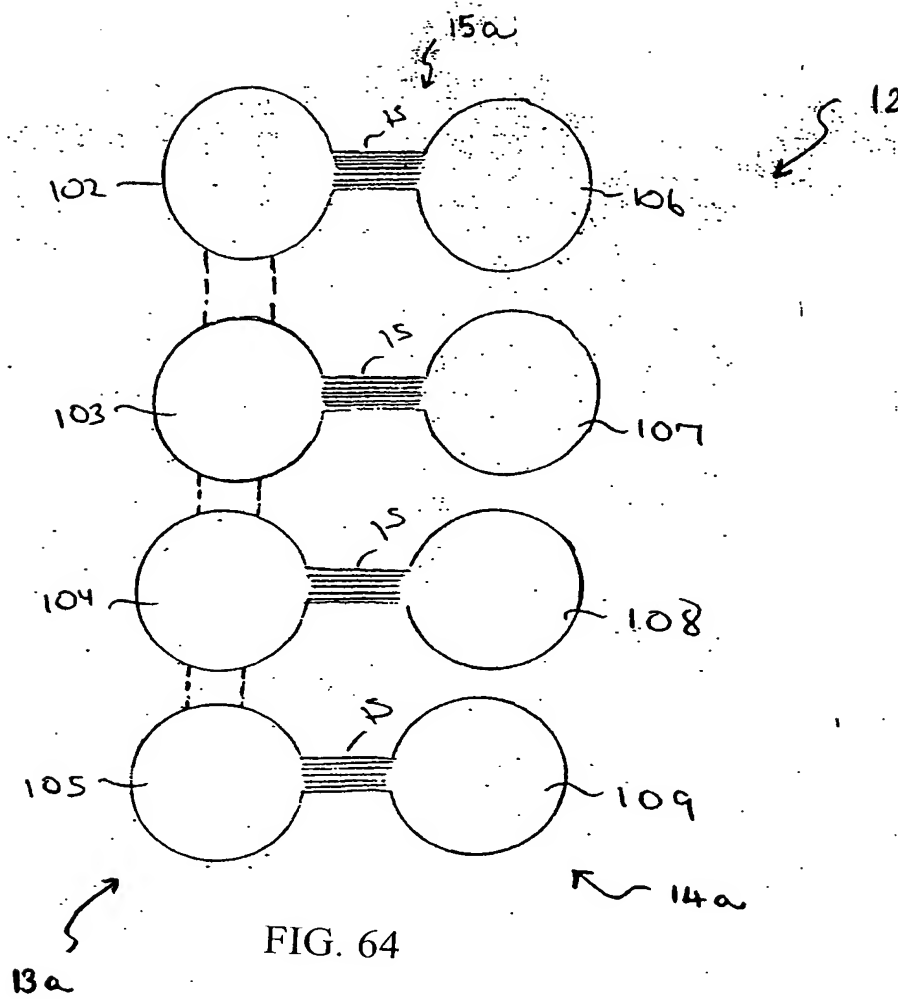


FIG. 64

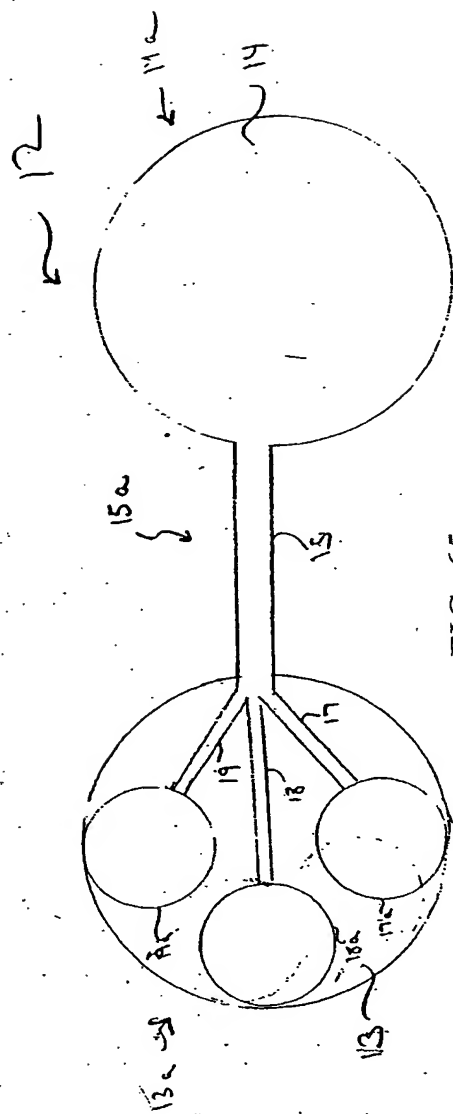


FIG. 65

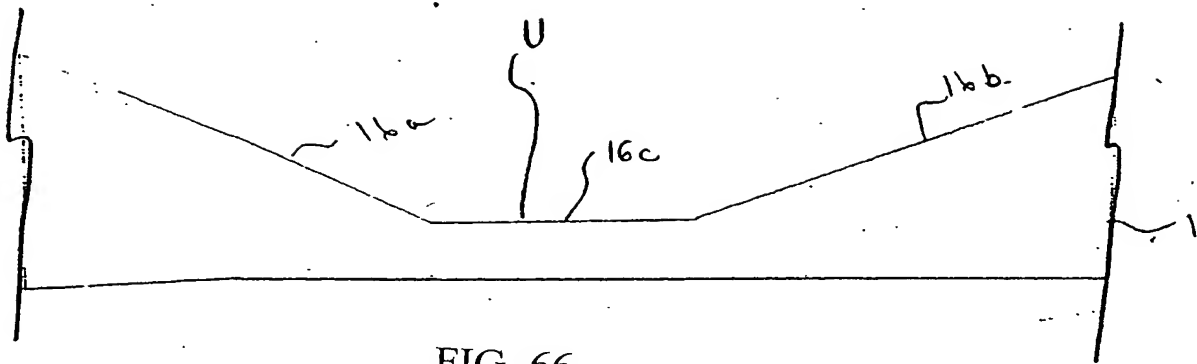


FIG. 66

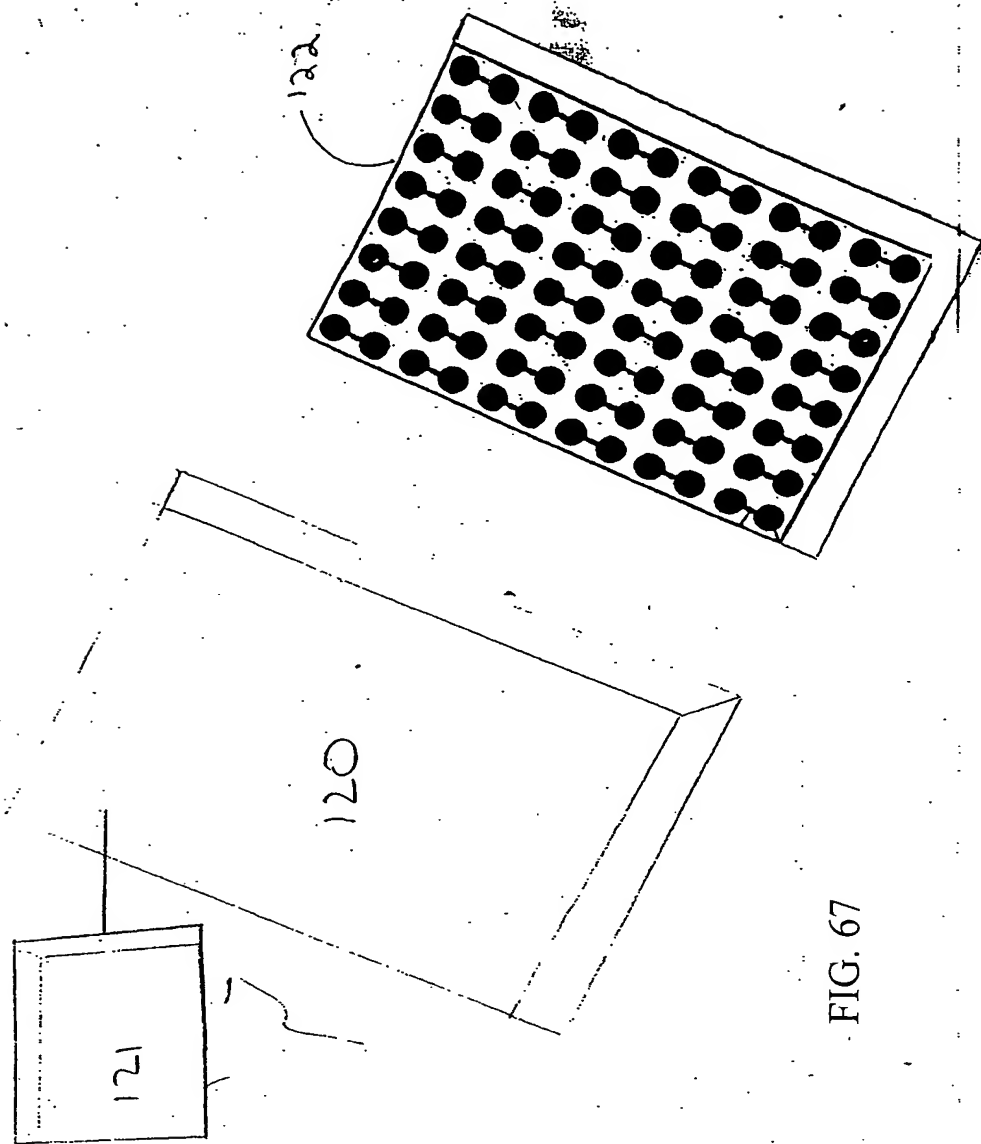


FIG. 67

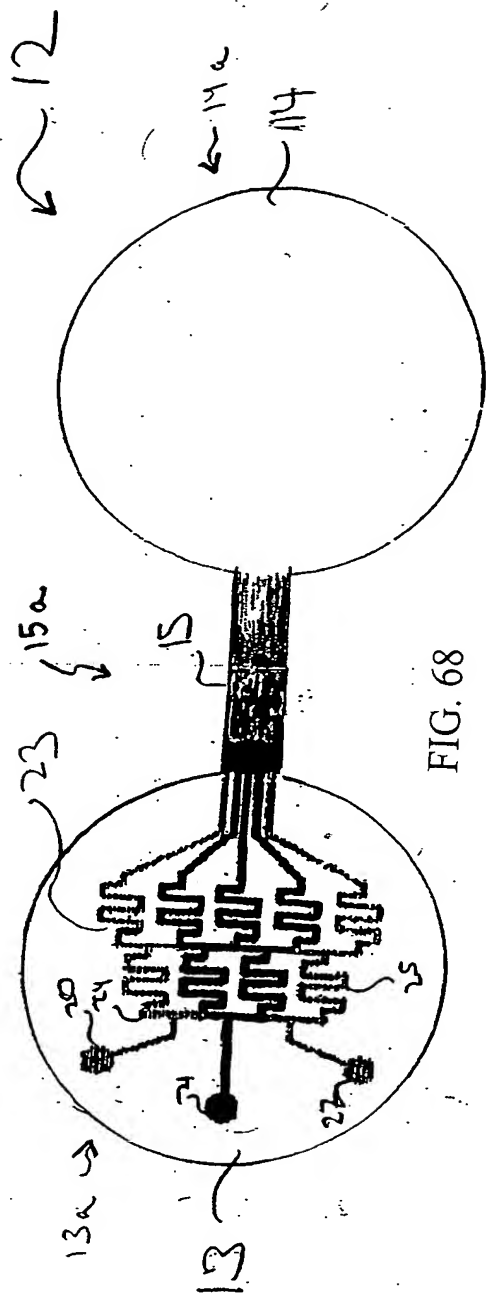


FIG. 68

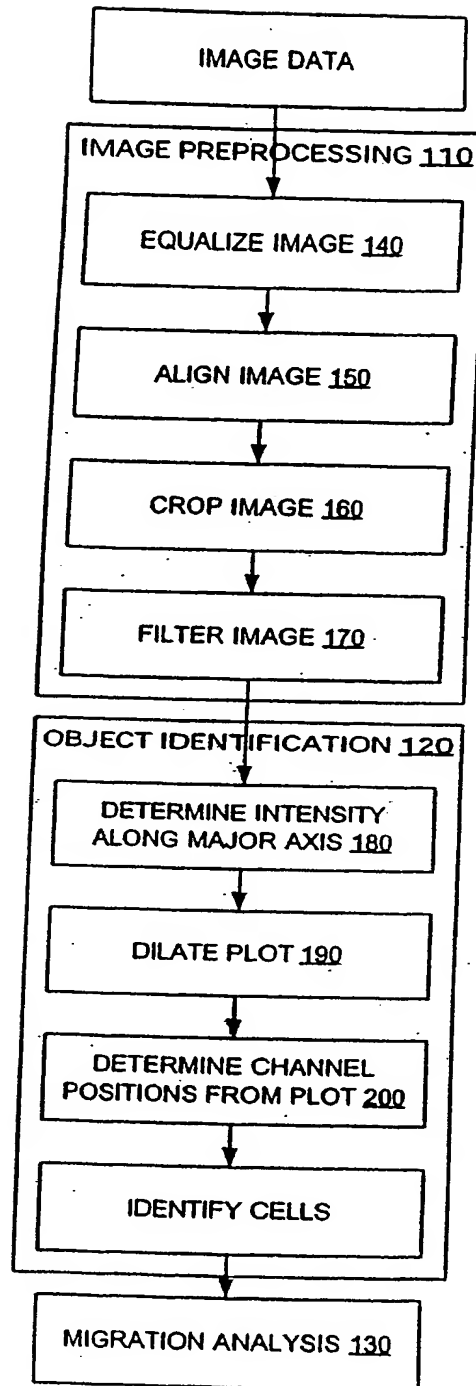
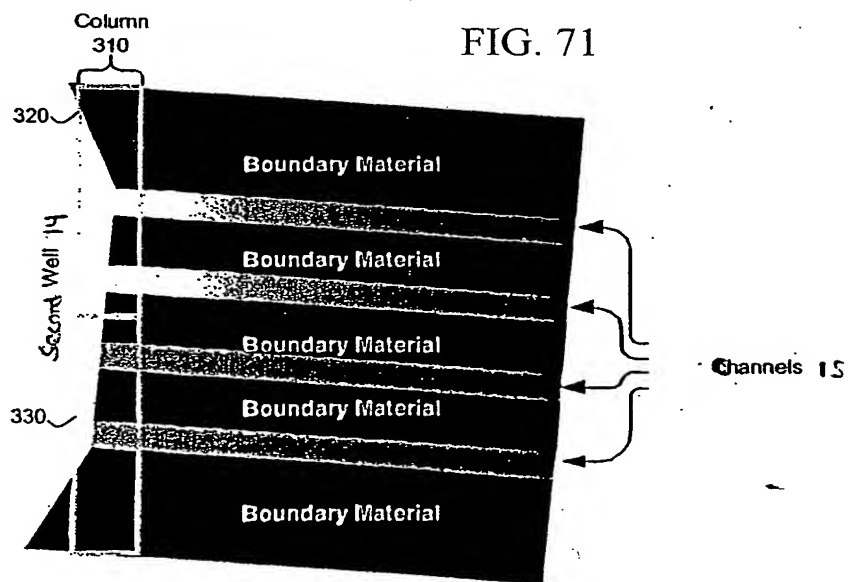
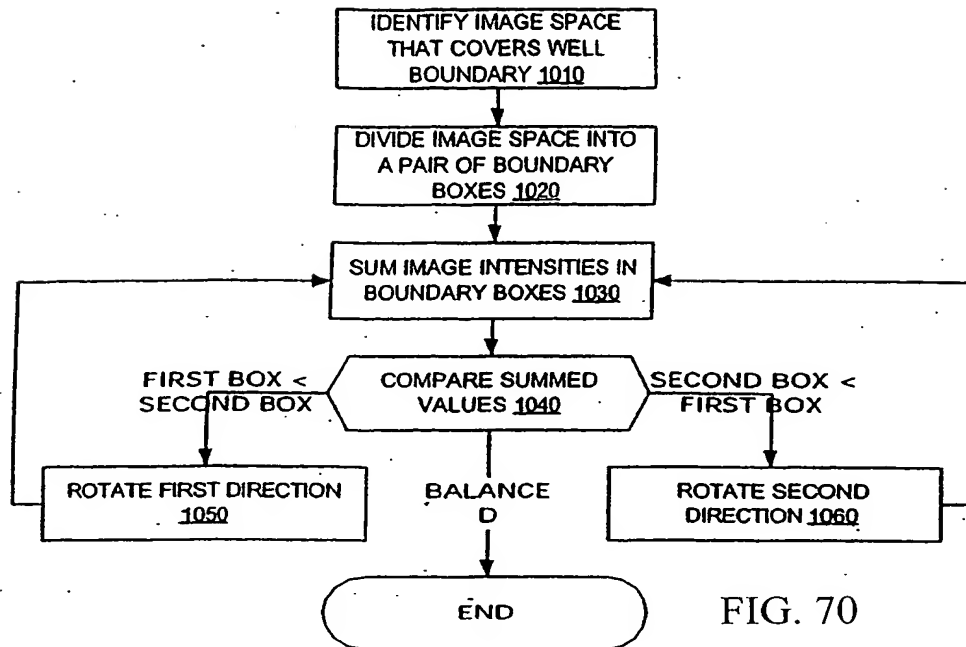
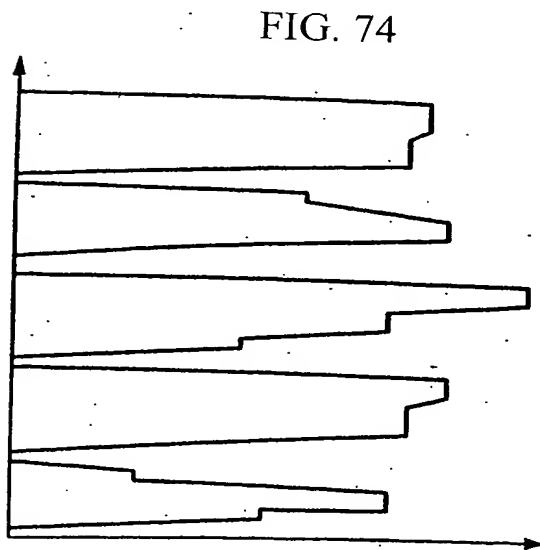
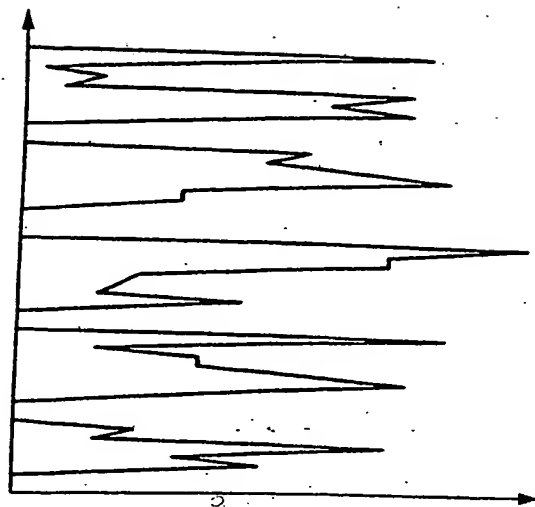
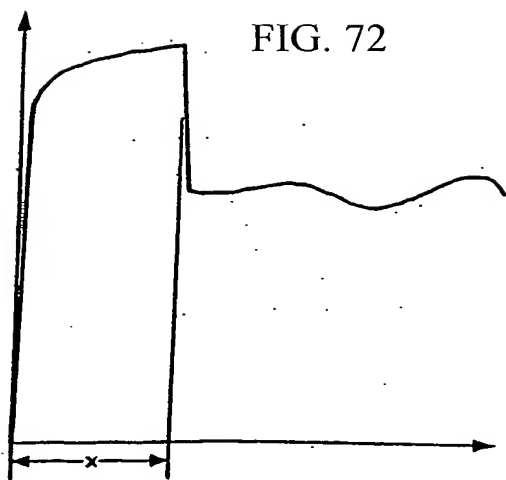


FIG. 69





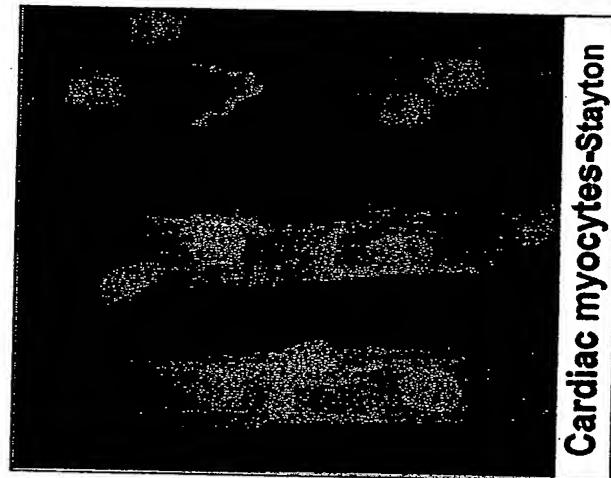
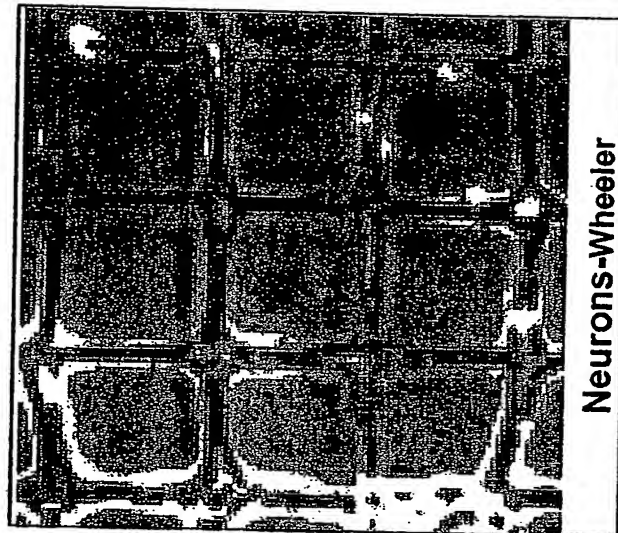
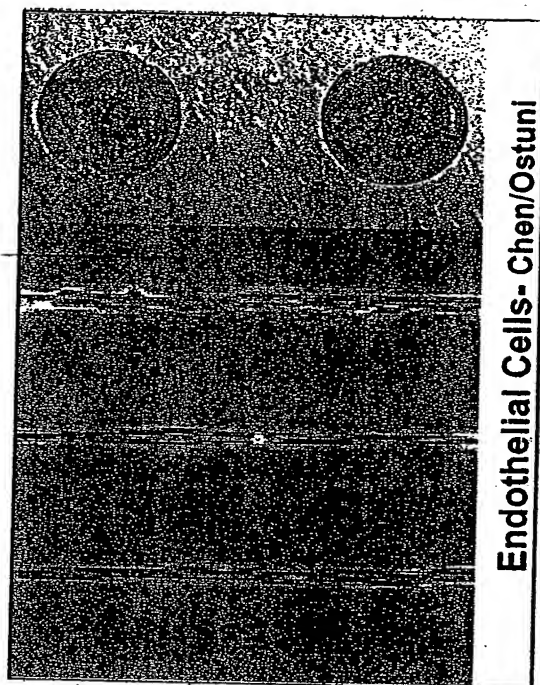
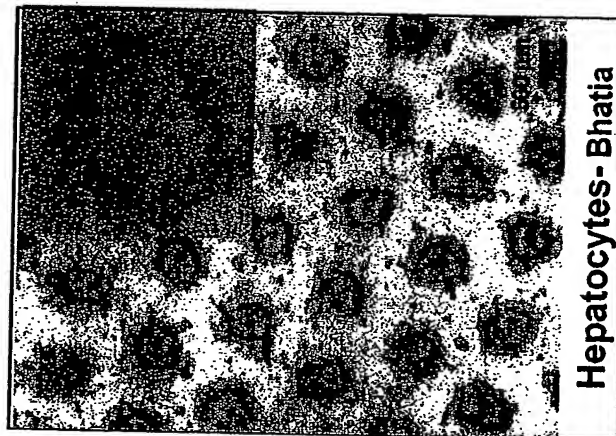


FIG. 75

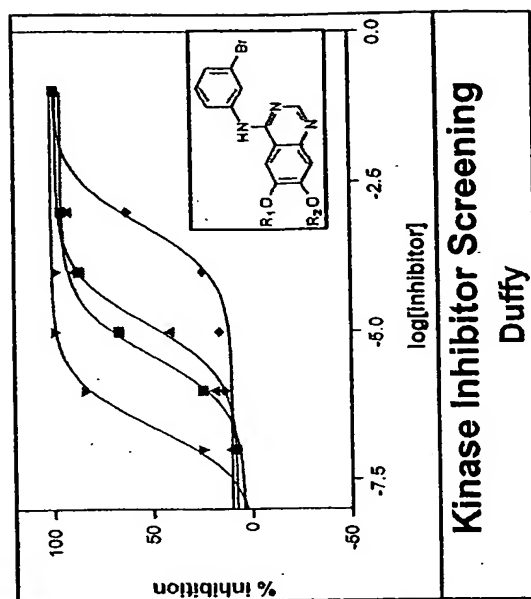
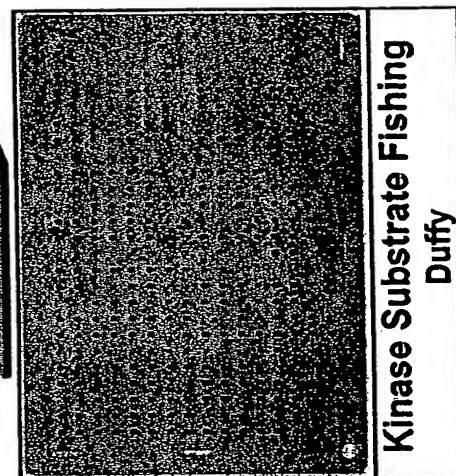
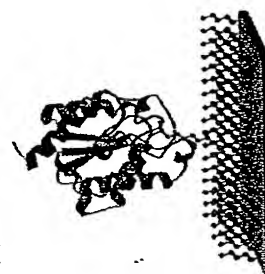
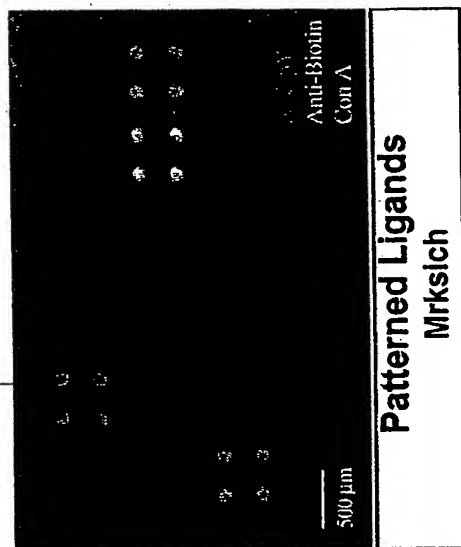
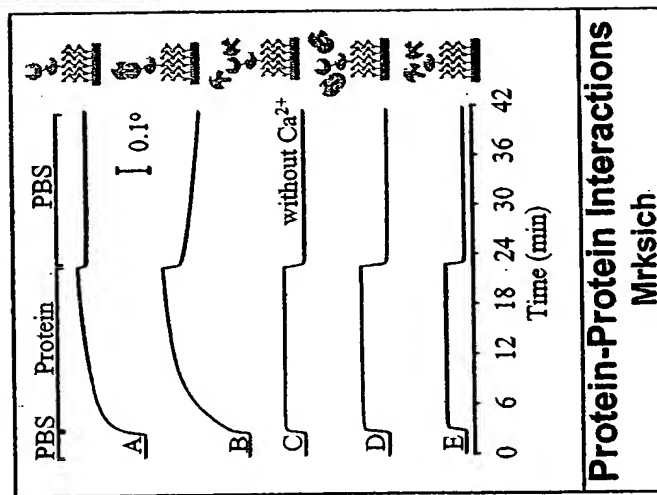
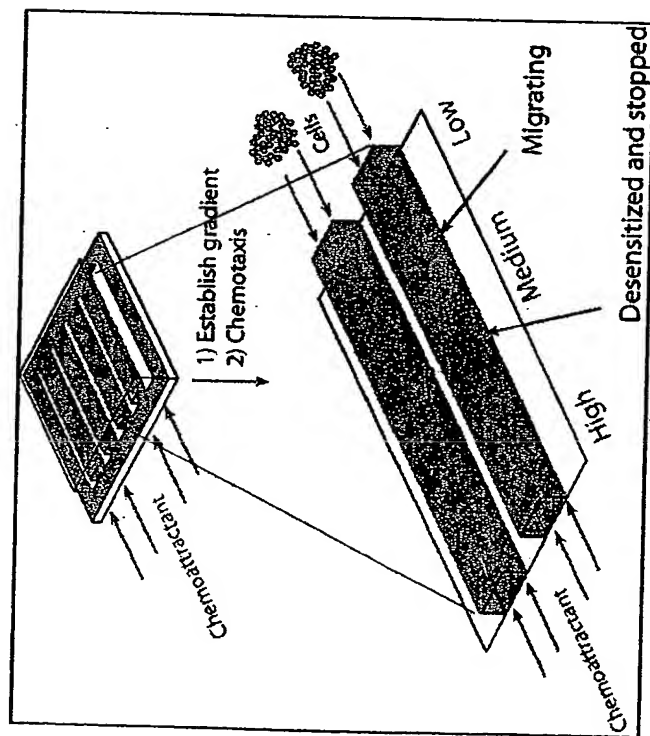


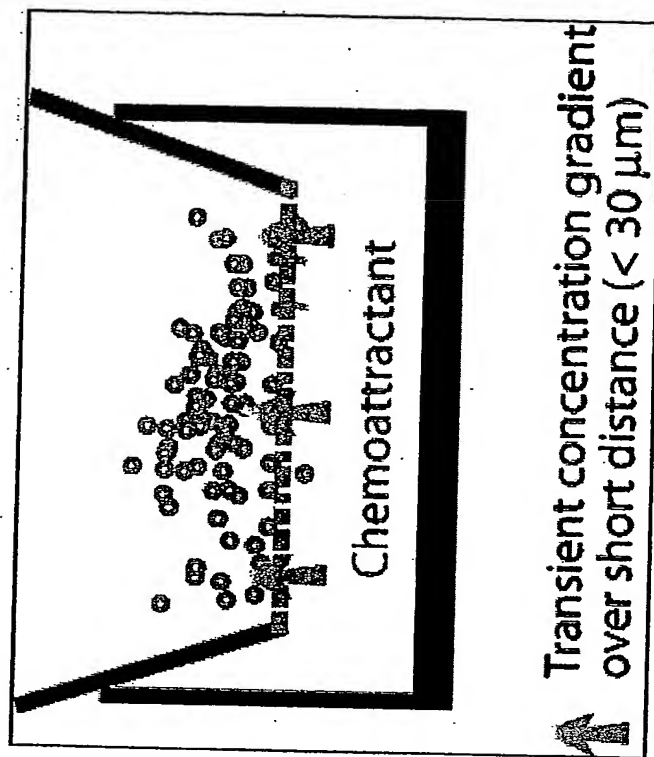
FIG. 76



Surface Logix

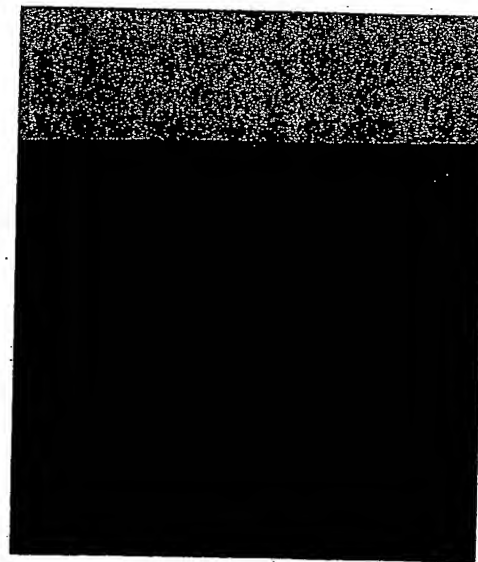
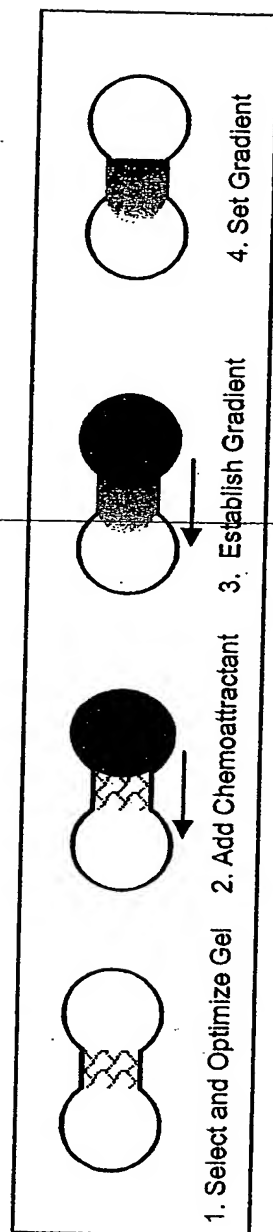
+ + + + + + + +

Stable chemical gradient
Linear chemical gradient
Gradient diversity (composition/size)
Quantifiable gradient
Real time monitoring
Distance traveled and density
Cell morphology

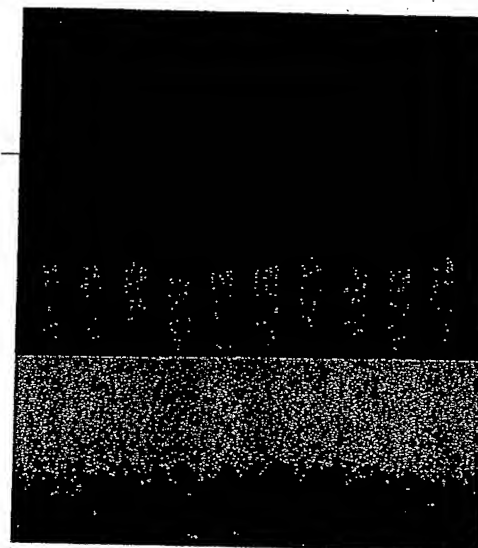


Transwell

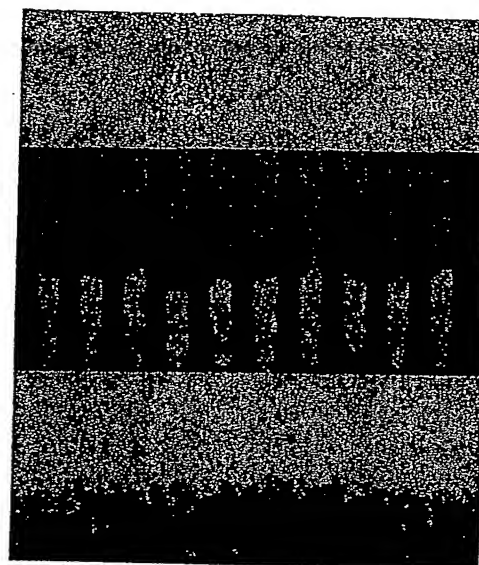
- - - - -



Gradient Formation
(MCP-1 + dye)



Migrating Cells (THP-1)



Robust Data

FIG. 78

Rhodamine-Dextran
(MW=10 KDa)

1 mm

Hydrogel-filled
channels

Intensity analysis

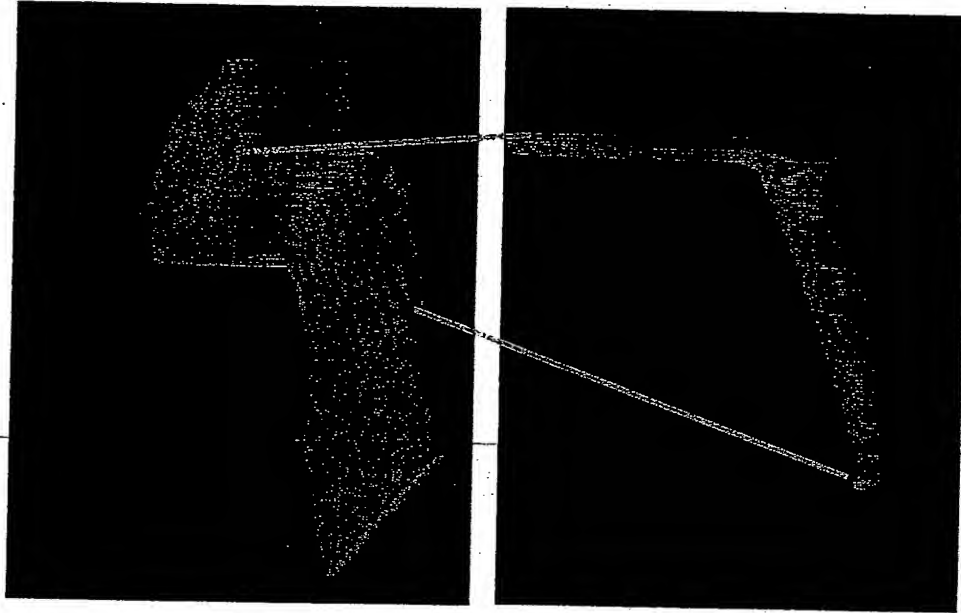
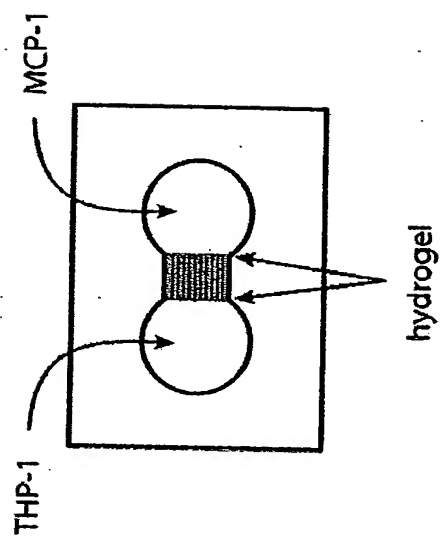


FIG. 79

Concentration at any point in the channel is calculated from either linear or logarithmic best curve fit



V: 25-50 μ L
 Cell number: 50,000/assay
 Time: 8-16 hrs

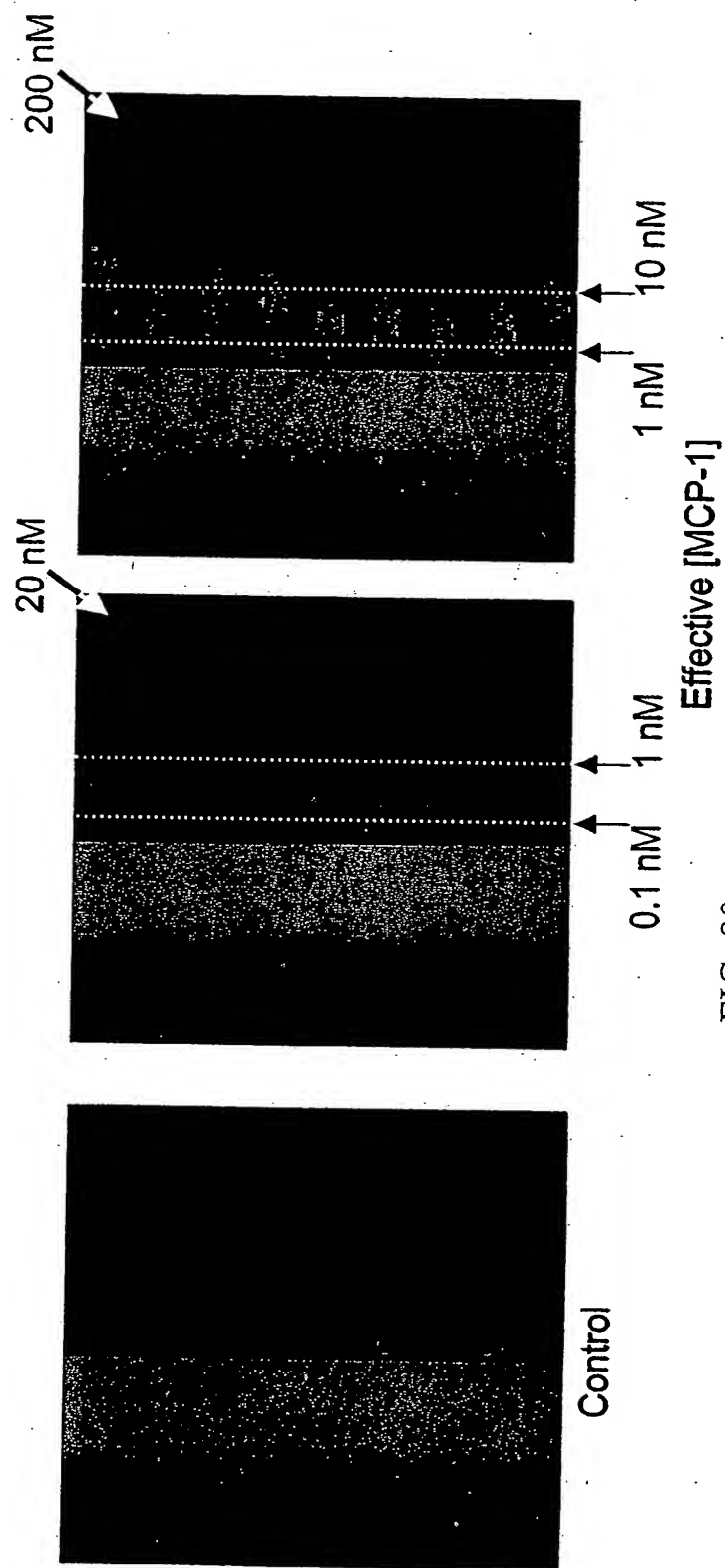


FIG. 80

THP-1 Cells and MCP-1 (10nM)

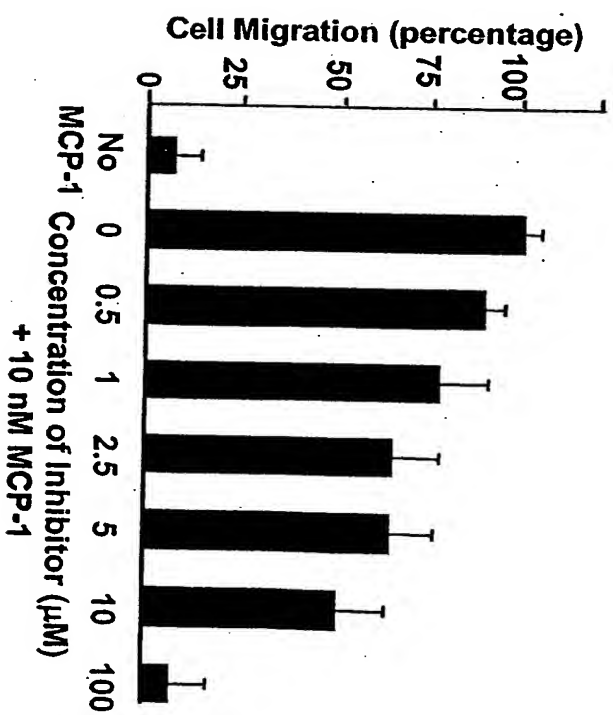
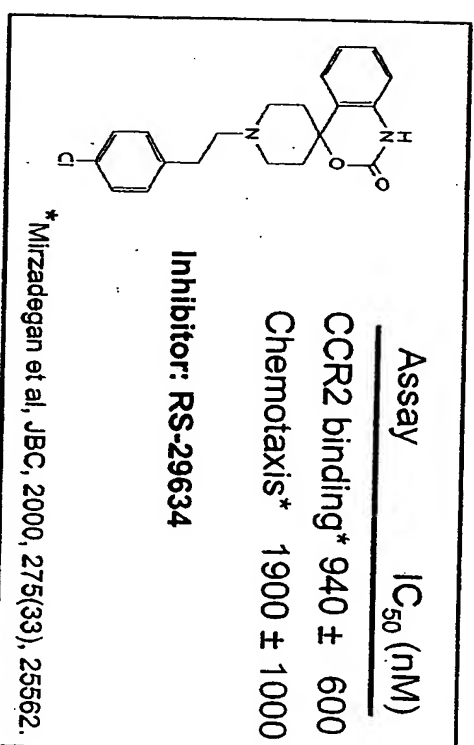
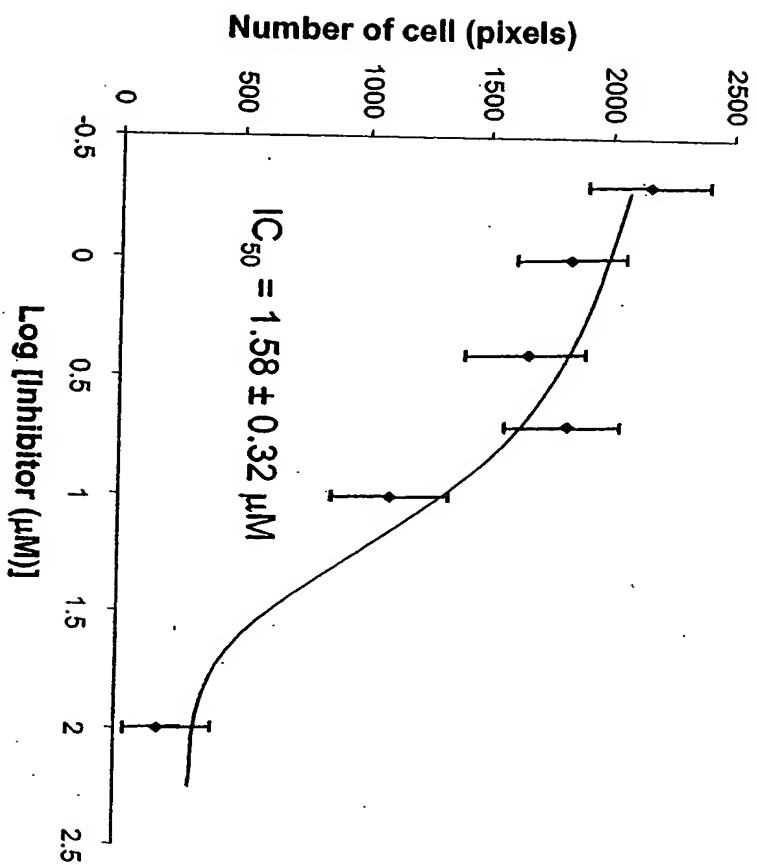


FIG. 81

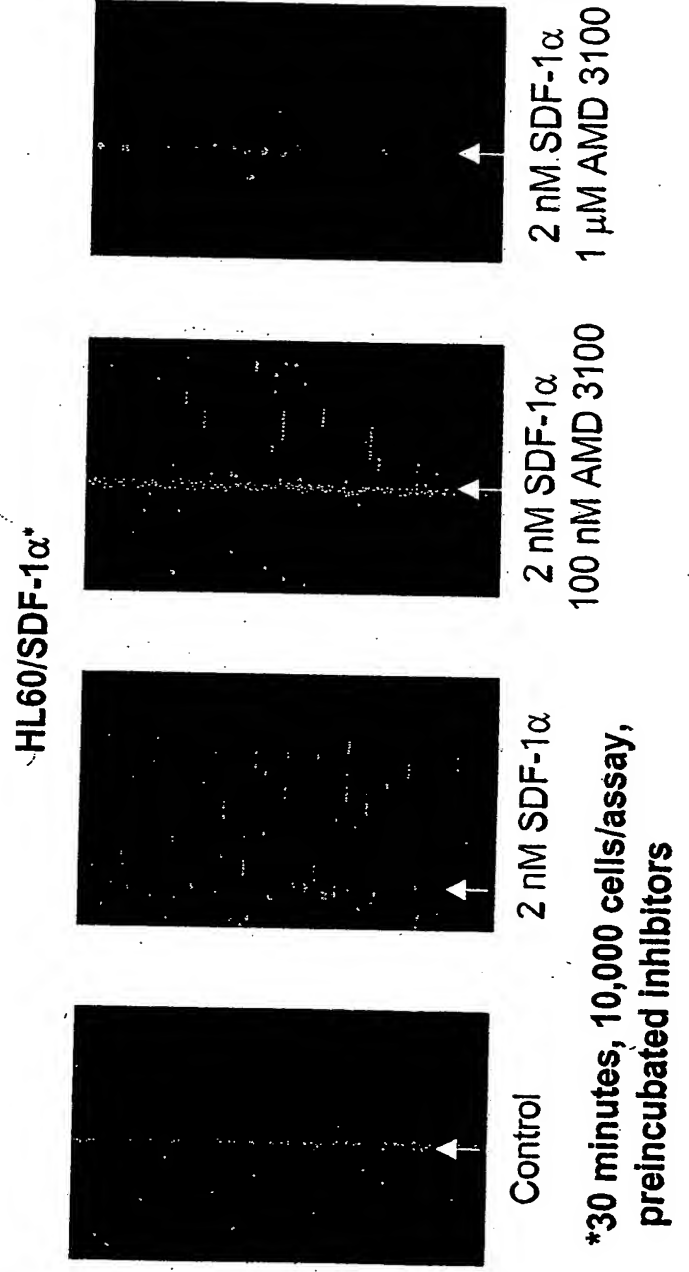
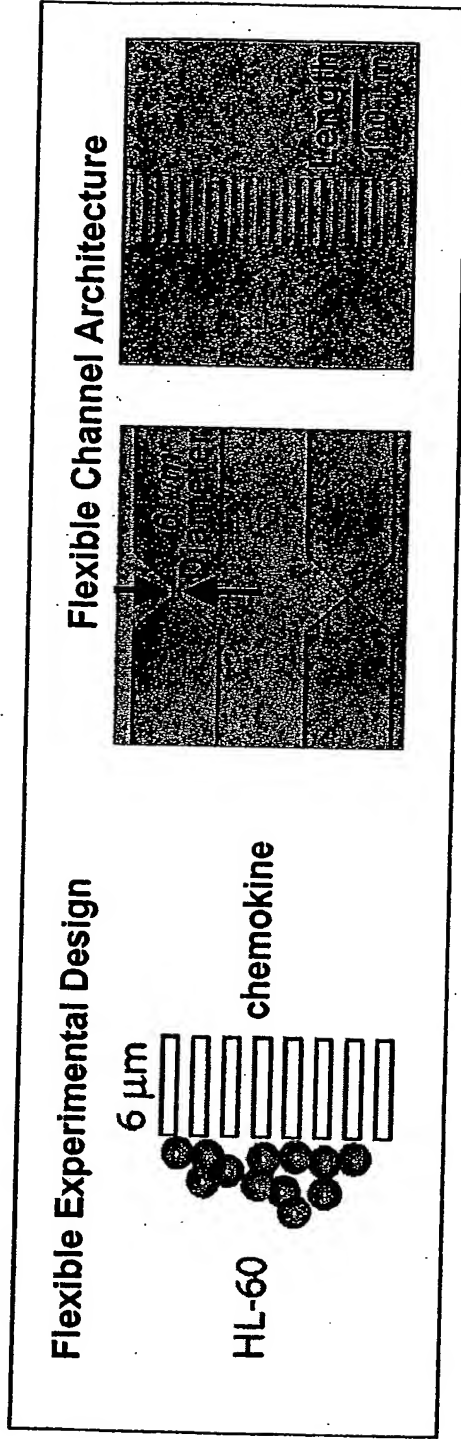
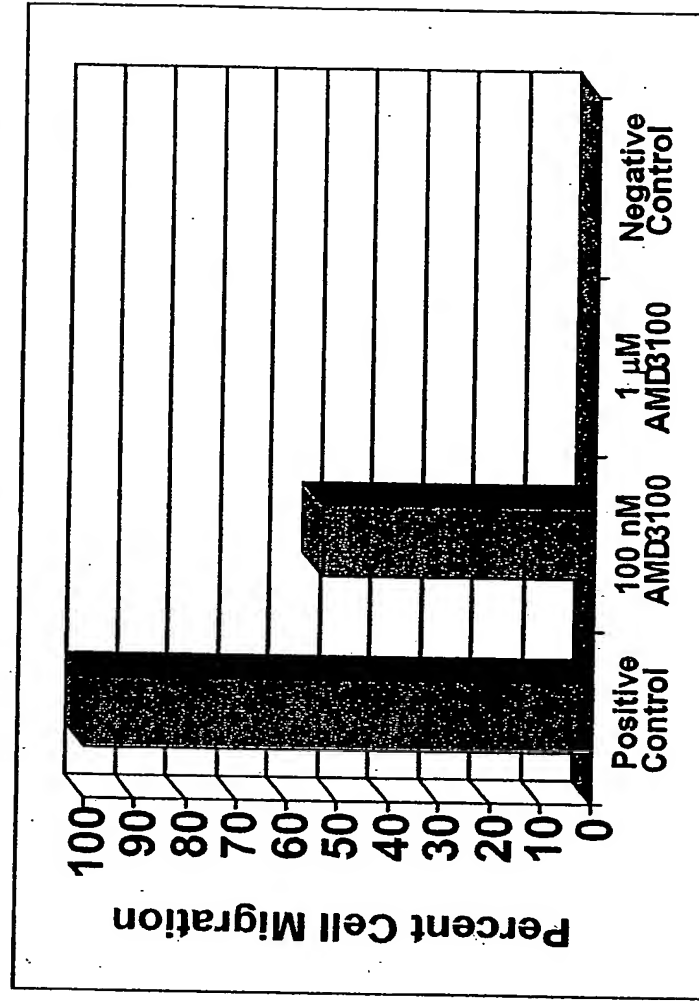
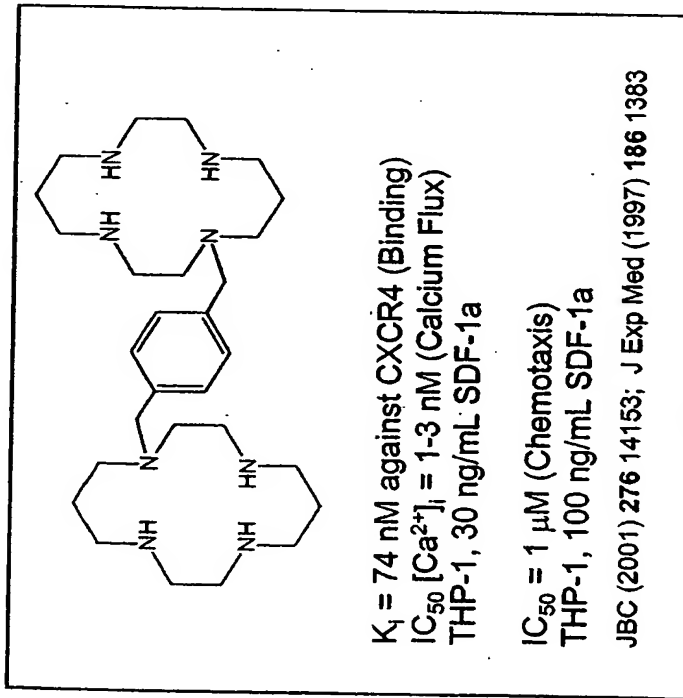
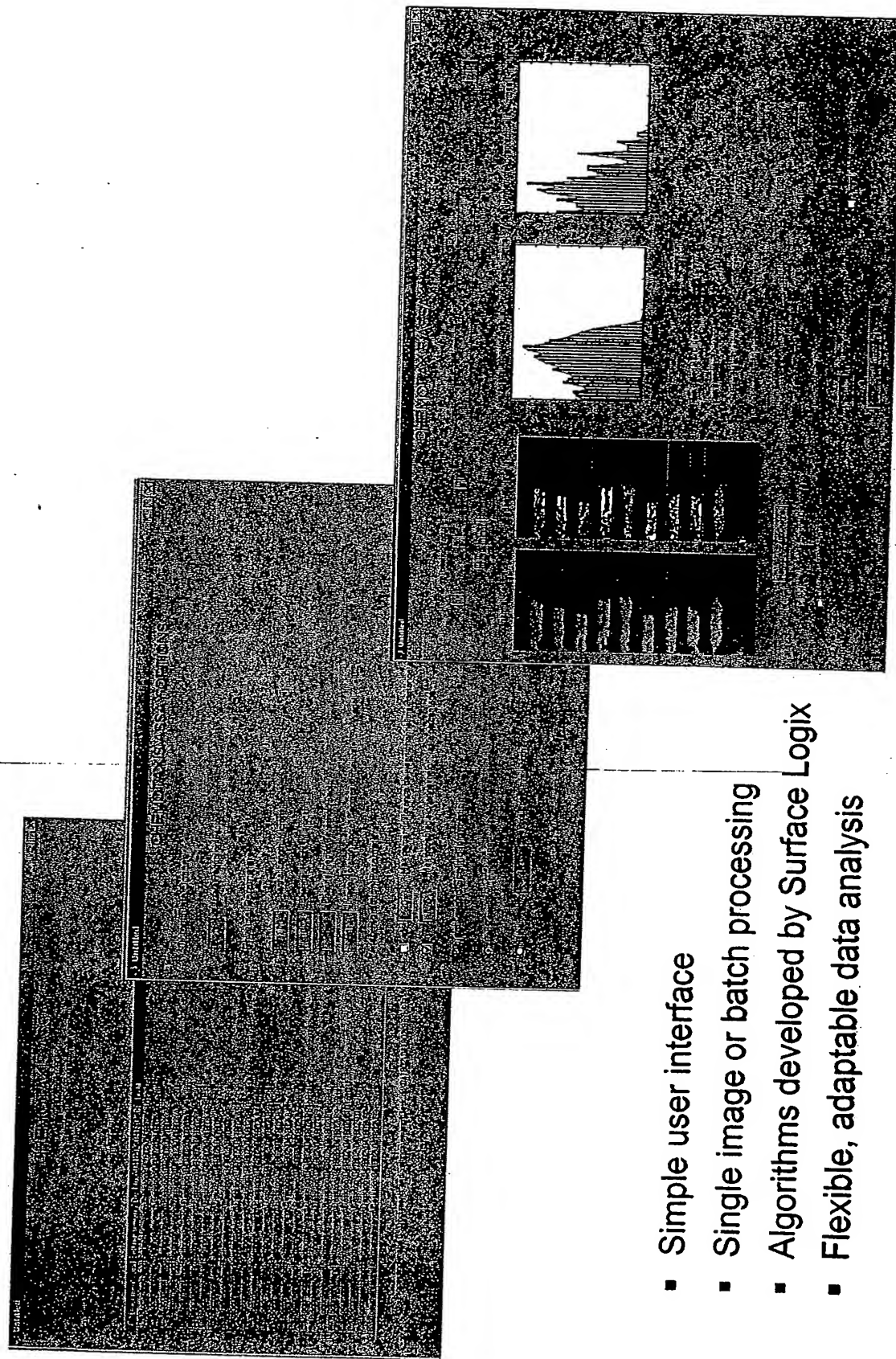


FIG. 82

HL60/SDF-1 α (2 nM, 30 minutes)



Proof of Principle Experiment (N=1 Cell Count Experiment)
Simplistic Data Analysis



- Simple user interface
- Single image or batch processing
- Algorithms developed by Surface Logix
- Flexible, adaptable data analysis

FIG. 84

Selective Activation of Endothelium

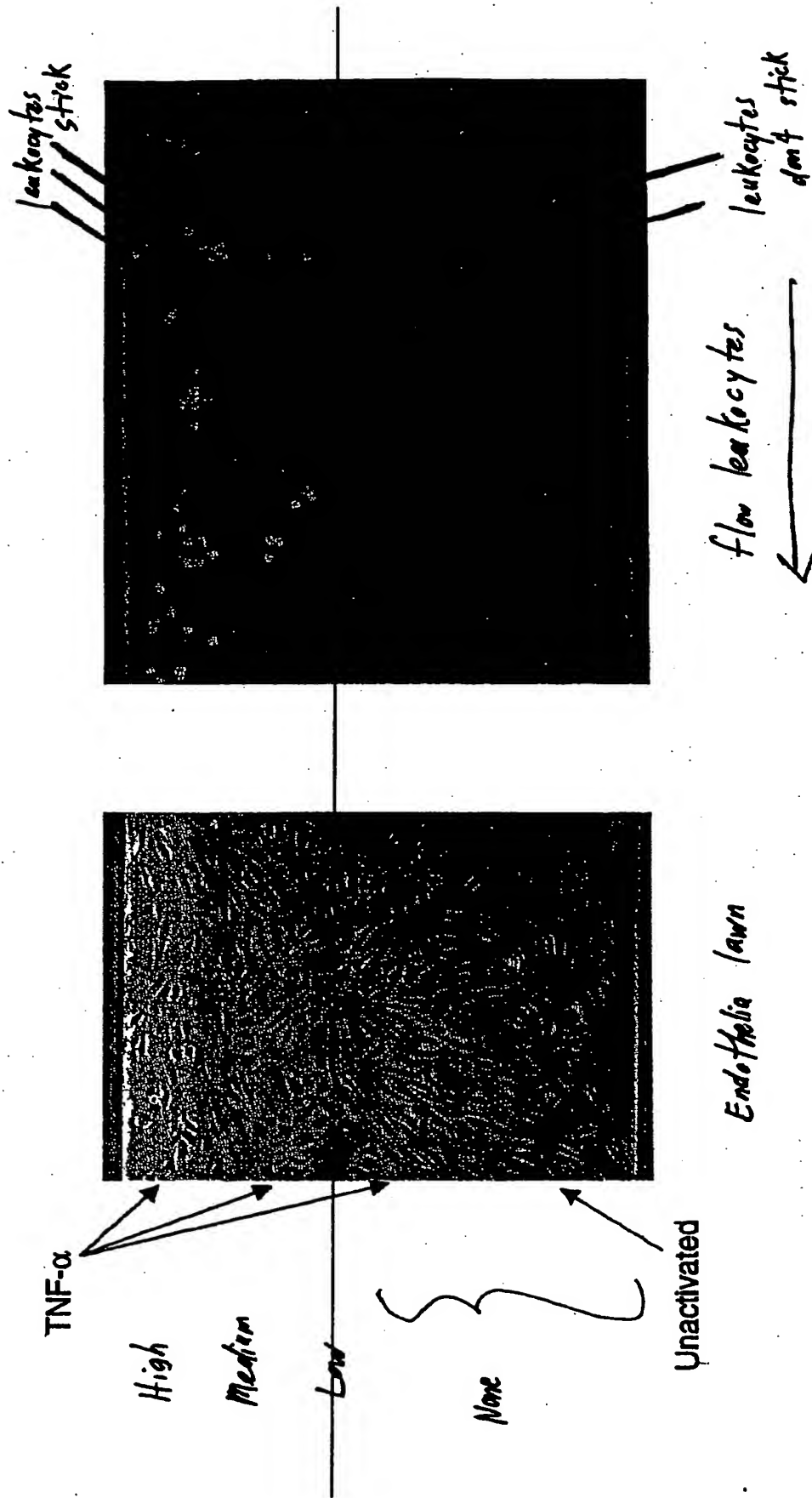


FIG. 85

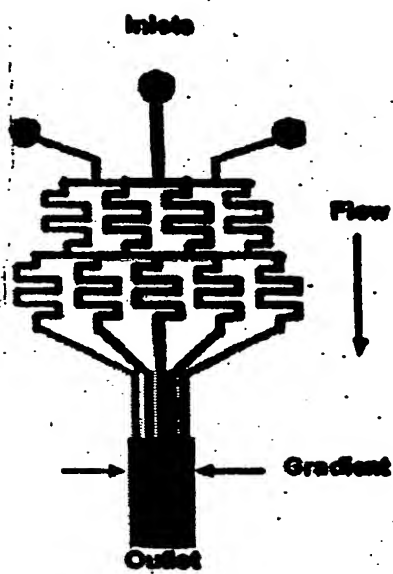


FIG. 86

Strategy and Opportunities

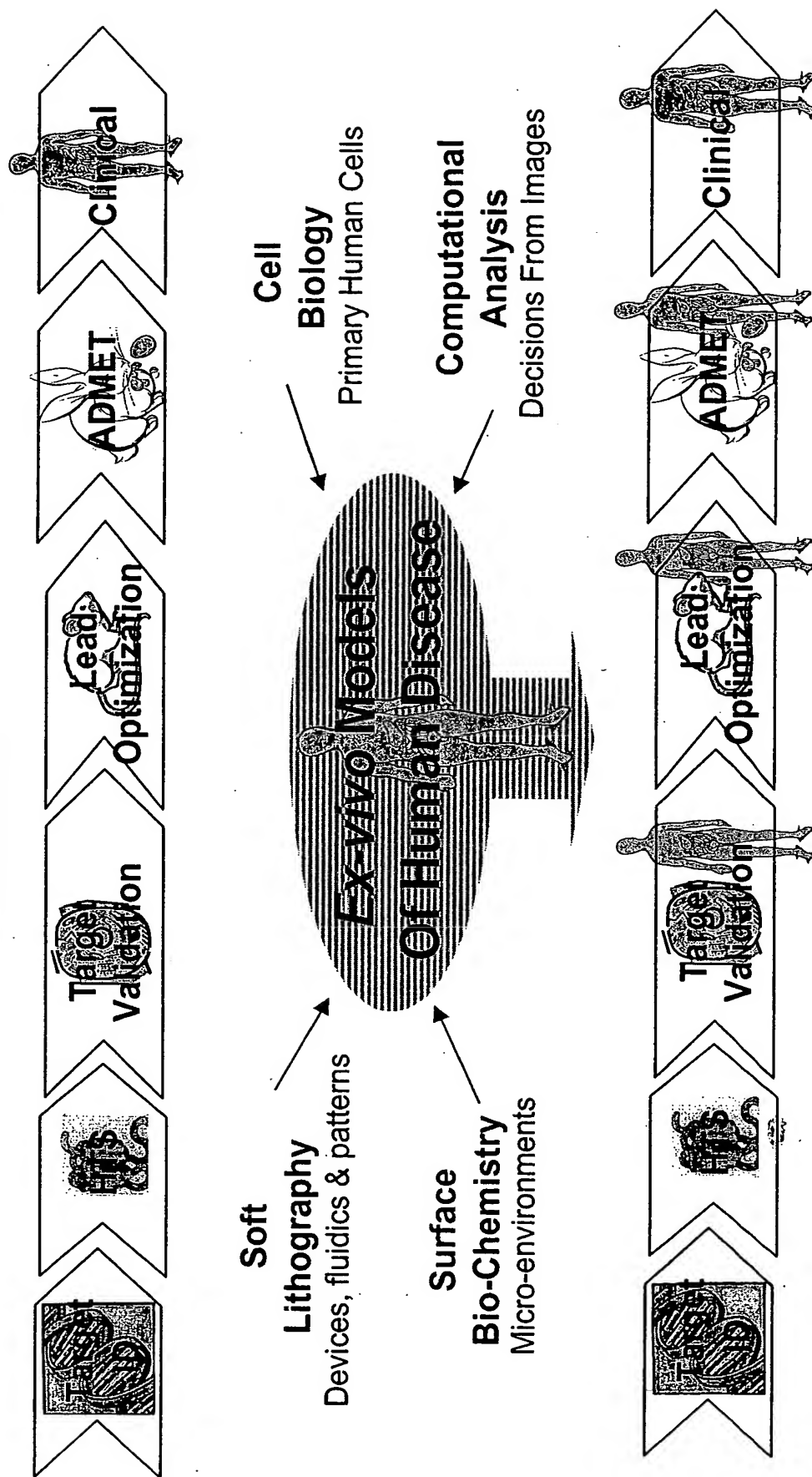


FIG. 87

Creating Subject Profiles

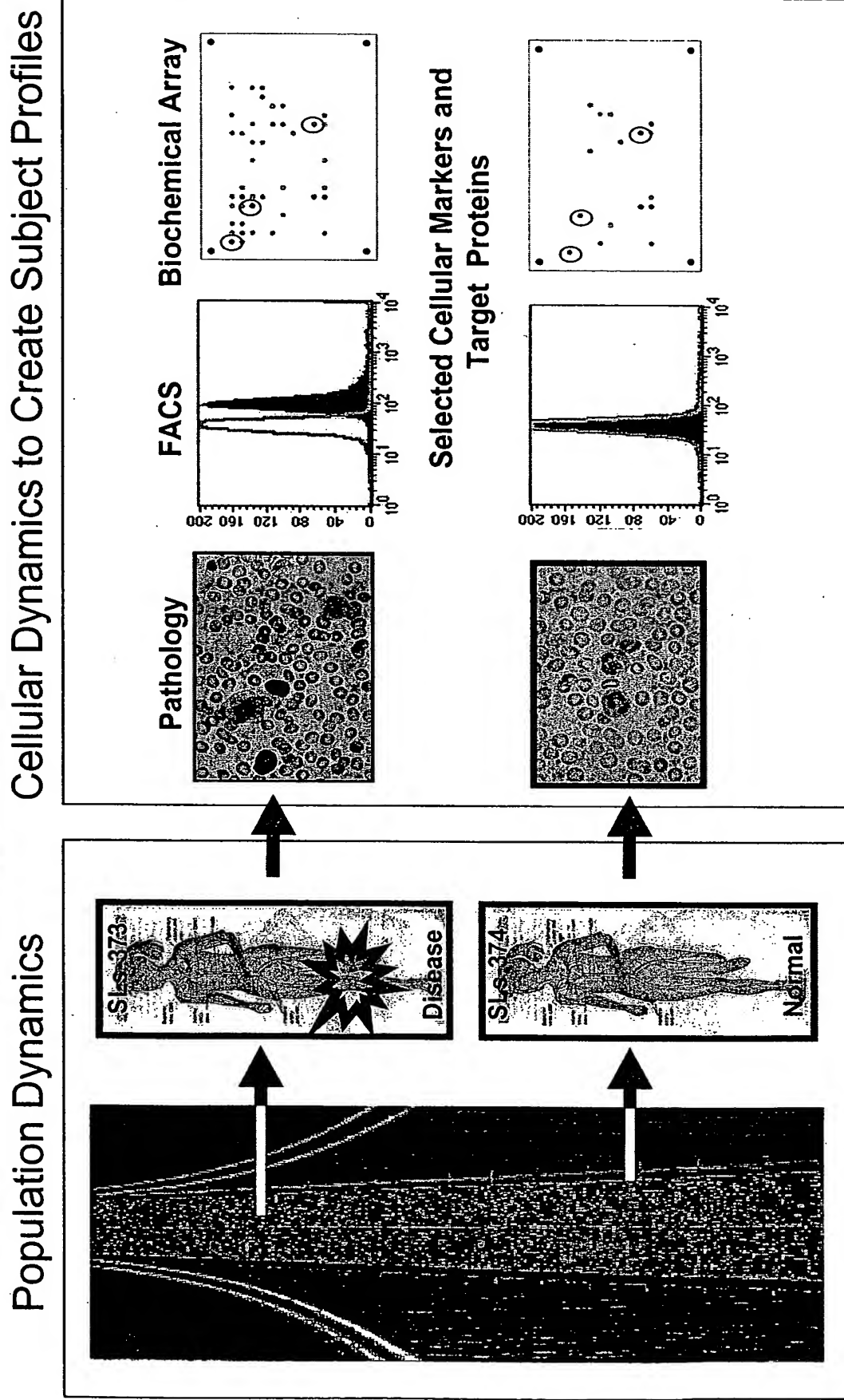


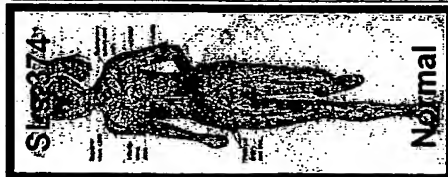
FIG. 88

Pharmacological Response

Subject Profiles



FACS	SLs-373	
	PBMCs	Monocytes
CD14	64%	89%
CD11b (MAC-1)	86%	93%
CD62L (L-selectin)	90%	89%
Target 1	39%	40%
Target 2	75%	92%
Target 3	2%	1%
Target 4	6%	6%



FACS	SLs-374	
	PBMCs	Monocytes
CD14	64%	89%
CD11b (MAC-1)	86%	93%
CD62L (L-selectin)	90%	89%
Target 1	37%	44%
Target 2	5%	8%
Target 3	1%	1%
Target 4	3%	7%

Pharmacological Response




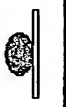




















Inflammation- Target 2			
Cellular Events	SLs-373	SLs374	
			
			
			
			
Rolling and Adhesion			
Transmigration	SLs-373	SLs374	
			
			
			
			
Chemotaxis			
SLs-373	SLs 001--XXXX	SLx 001--XXXX	SLx 001--XXXX
	SLx 002--XXXX	SLx 002--XXXX	SLx 002--XXXX
	SLx 003--XXXX	SLx 003--XXXX	SLx 003--XXXX
	SLx 004--XXXX	SLx 004--XXXX	SLx 004--XXXX
	SLx 005--XXXX	SLx 005--XXXX	SLx 005--XXXX

FIG. 89

Linking Target Expression to Compound Activity- Preclinical

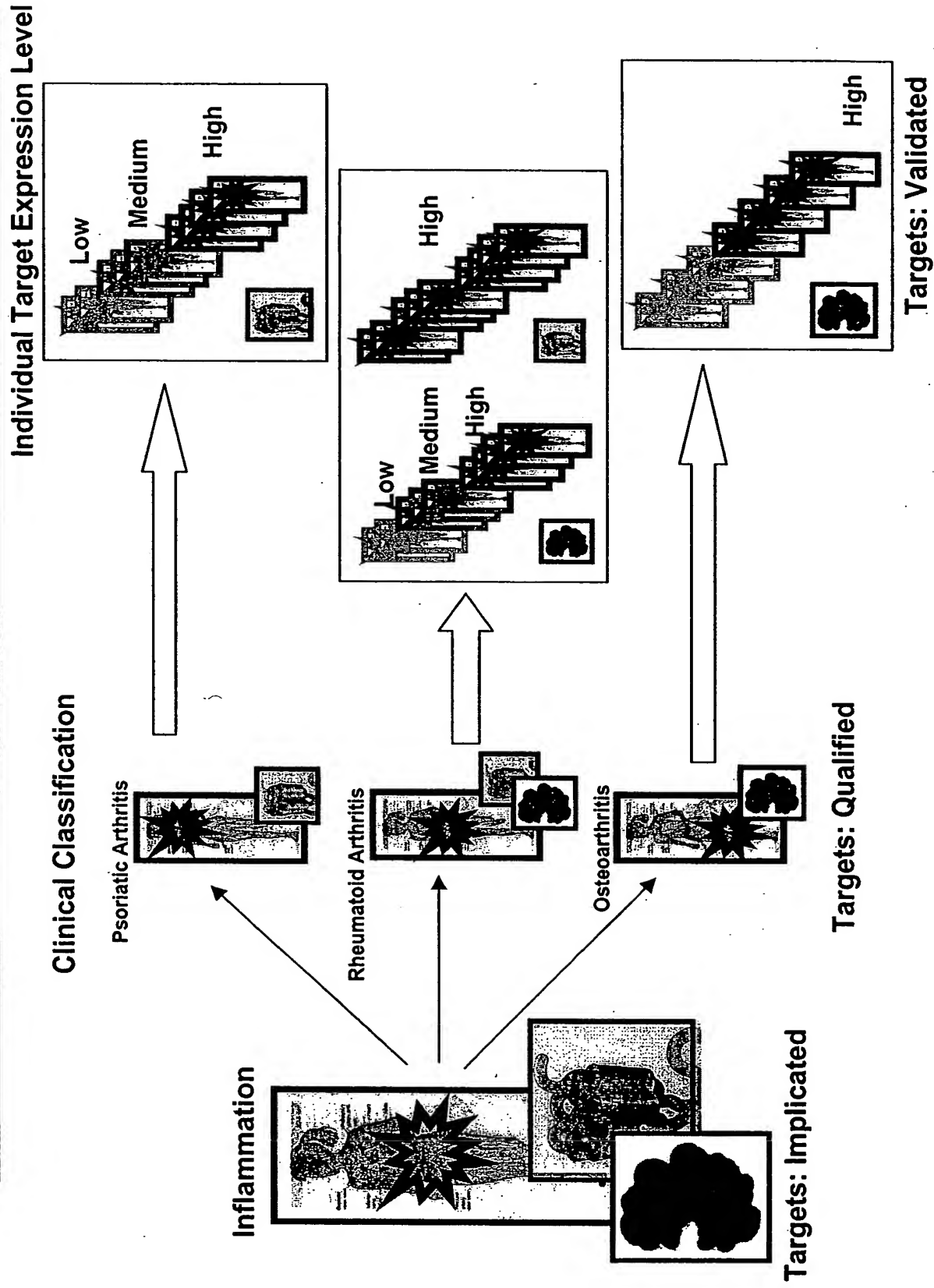


FIG. 90

Linking Compound Activity to Subject Profile- Preclinical

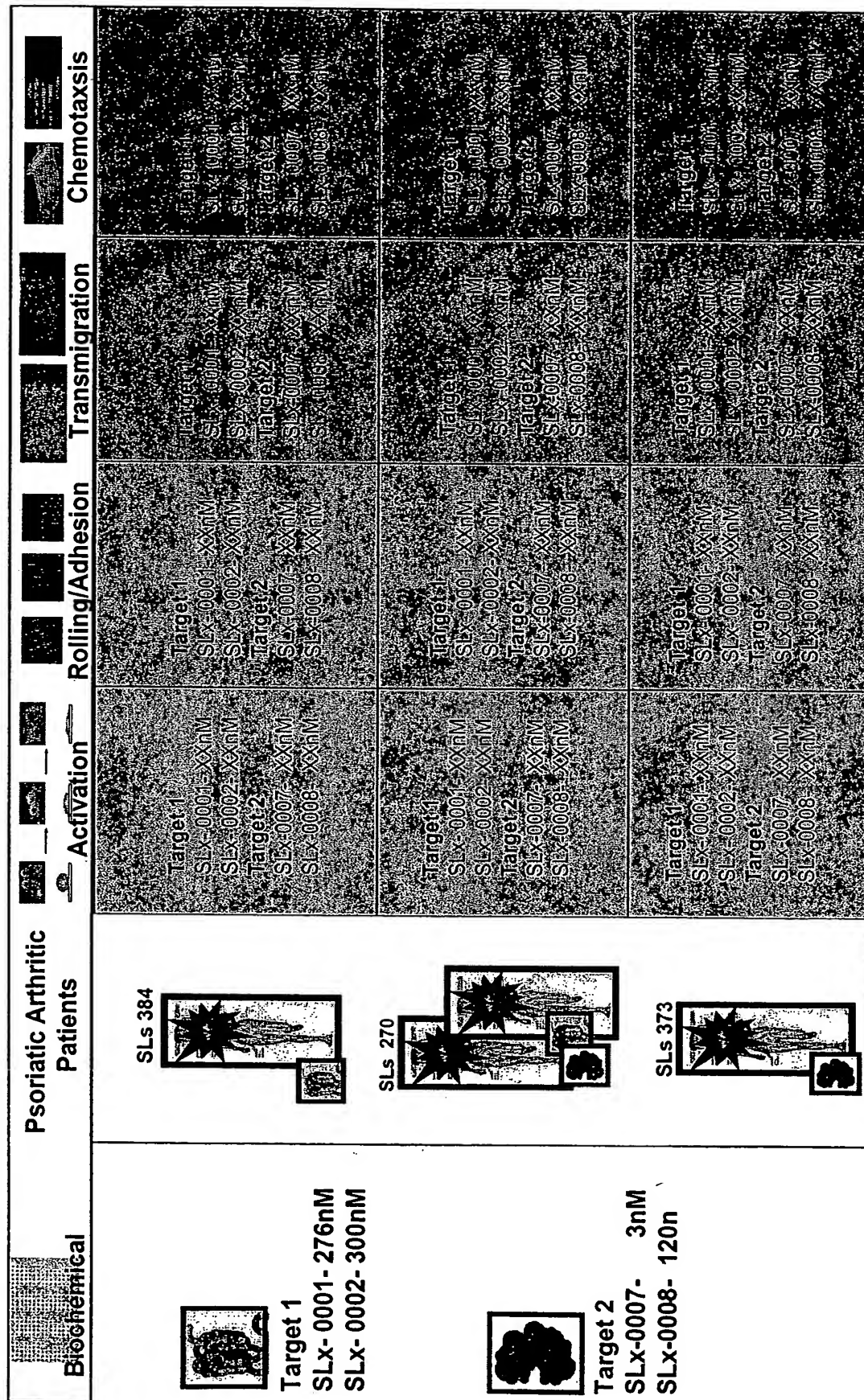
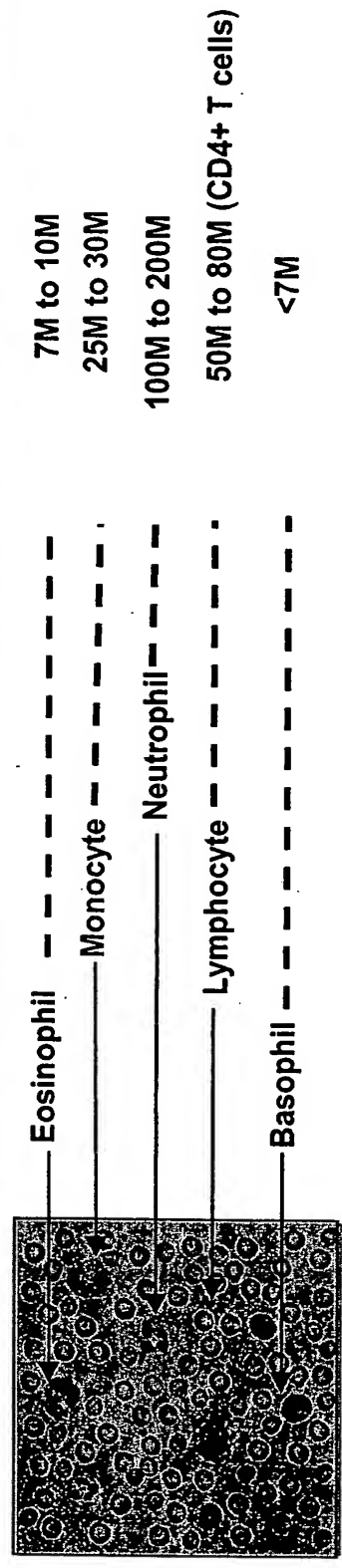


FIG. 91

Targeting Inflammation Disease: White Blood Cells

Isolation (Control Subjects): Average Cell Yield Per Unit



Monocyte Utilization Average DATA Points Per Unit Signal to Noise

Traditional Transwell (Corning) 60 total pt 5 to 1



Cells Per Well: 500,000 to 1M

SLx Diapedesis Assay



Cells Per Unit: 25,000 to 50K

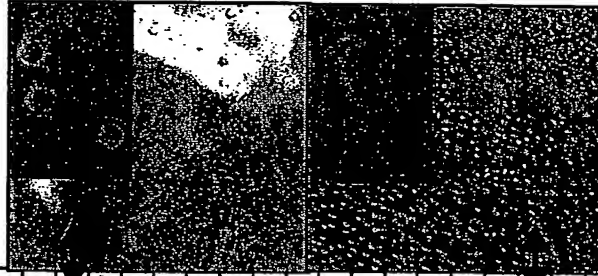
1,200 pt 15 to 1

20pt- Total/Blanks
1,080pt- 60 x 6pt. IC50 in triplicate

Creating a Control Environment

Monocyte Surface Treatments

	Surface	Contact Angle	Adhesion	Activation
Hydrophobic	SLX-C03	102.15	Med	Med
	SLX-C05	114.8	Med	Med
	SLX-C10	89.25	Med	Low
	SLX-C34	103.15	Med	Med
	SLX-C28	111.25	Low	Low
	SLX-C29	90.15	Low	None/Low
	SLX-C26	93	Med	Med
	SLX-C12	97.7	Med	Med
	SLX-C18	84.45	Med	High
	SLX-C25	73.4	High	High
Intermediate	SLX-C11	77.1	Med	Med
	SLX-C06	78.6	High	High
	SLX-C21	78.4	High	High
	SLX-C22	67.4	High	High
	SLX-C30	67.5	Low	Med
	SLX-C01	65.25	High	High
	SLX-C27	61.15	Med	High
	SLX-C24	60.95	Med	High
	SLX-C07	47.7	High	High
	SLX-C20	52.5	High	Med
Hydrophilic	SLX-C02	32.05	Med	Med
	SLX-C23	17.5	Med	Low
	SLX-C19	n/d	Med	High
	SLX-C13	n/d	Med	Med
	SLX-C14	n/d	Med	High
	Glass	n/a	High	High
	TC	n/a	High	High



Monocyte ECM Treatments

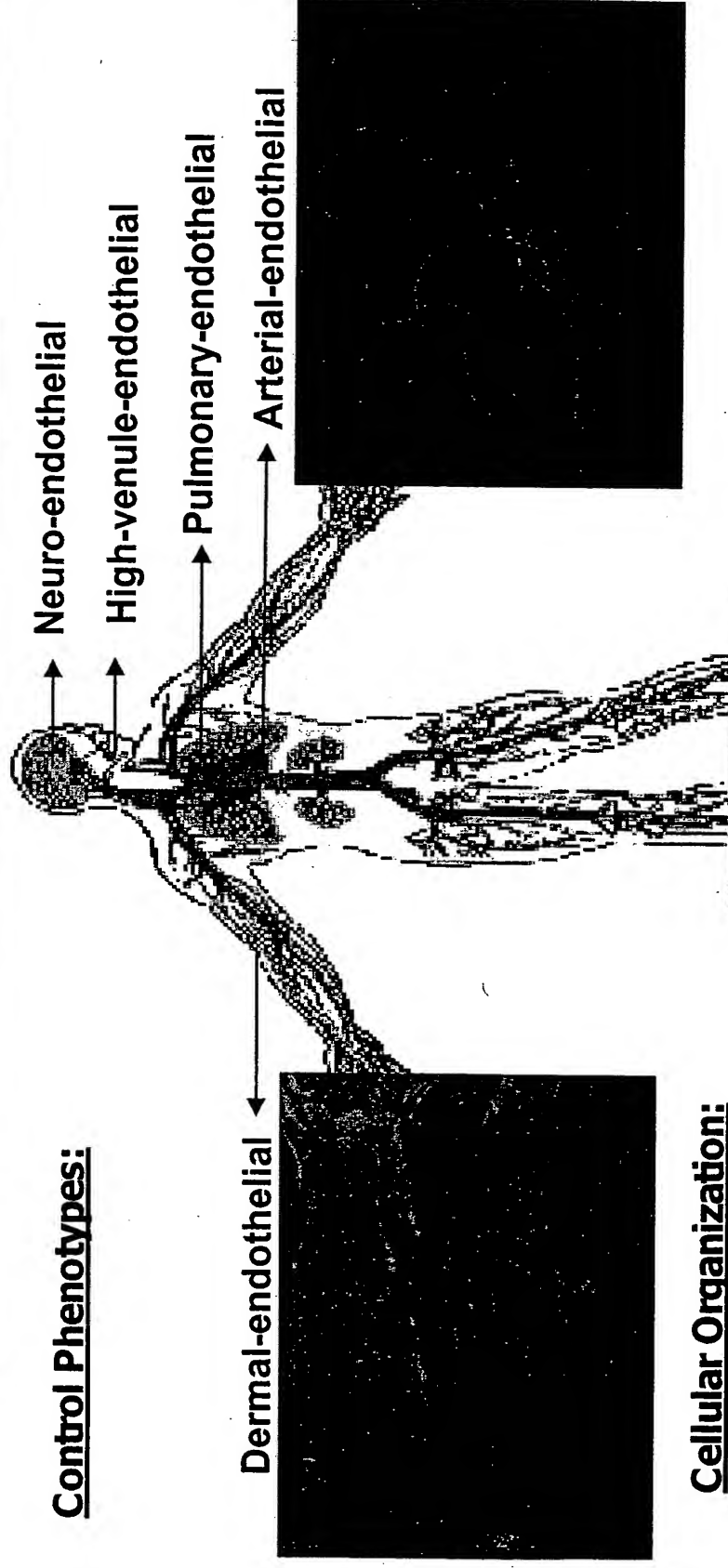
SLxG#	Primary Cells and Cell Lines				
	PMN	Monocyte	T-cell	Eosinophil	THP-1
SLxG110	X	X	X		✓
SLxG111	✓	X	X	X	✓
SLxG112	✓	✓	X	X	✓
SLxG113			X		
SLxG114		✓	X		✓
SLxG115		✓	↔	↔	↔
SLxG116					
SLxG117					X
SLxG118	✓	X	✓		X
SLxG119	✓	X	✓		X
SLxG120	✓	X	↔	↔	↔
SLxG121					X
SLxG122					X
SLxG123					X
SLxG124	✓	✓			
SLxG125	✓	✓	↔	↔	↔
SLxG126					
SLxG127					
SLxG128					X
SLxG129					✓
SLxG130				X	
SLxG131				X	
SLxG132					
SLxG133					X
SLxG134					X
SLxG135					X



FIG. 93

Targeting Inflammation Disease: Endothelial Cells

Control Phenotypes:



Cellular Organization:

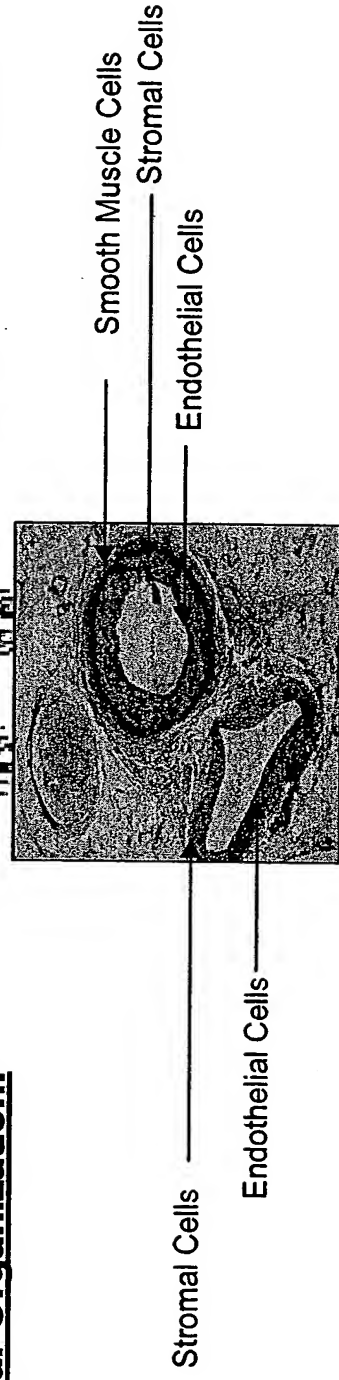
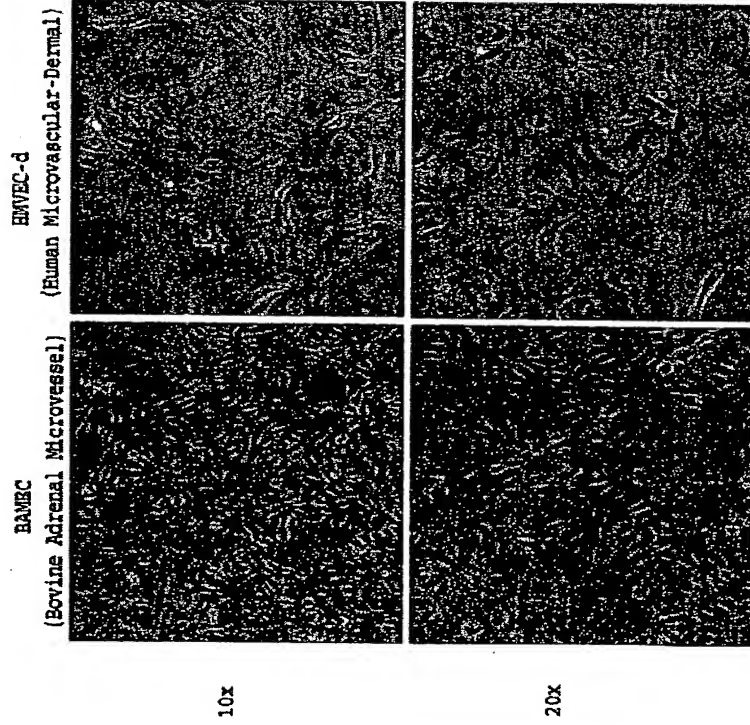


FIG. 94

Creating Capillary Like Structures

Traditional Cell Culture



SLx Cell Culture

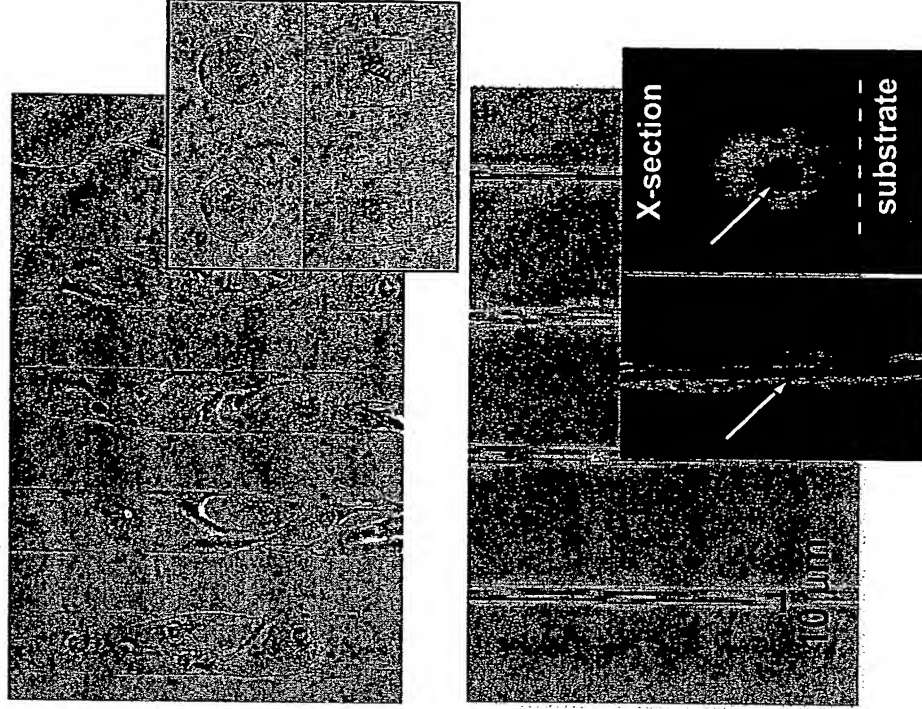
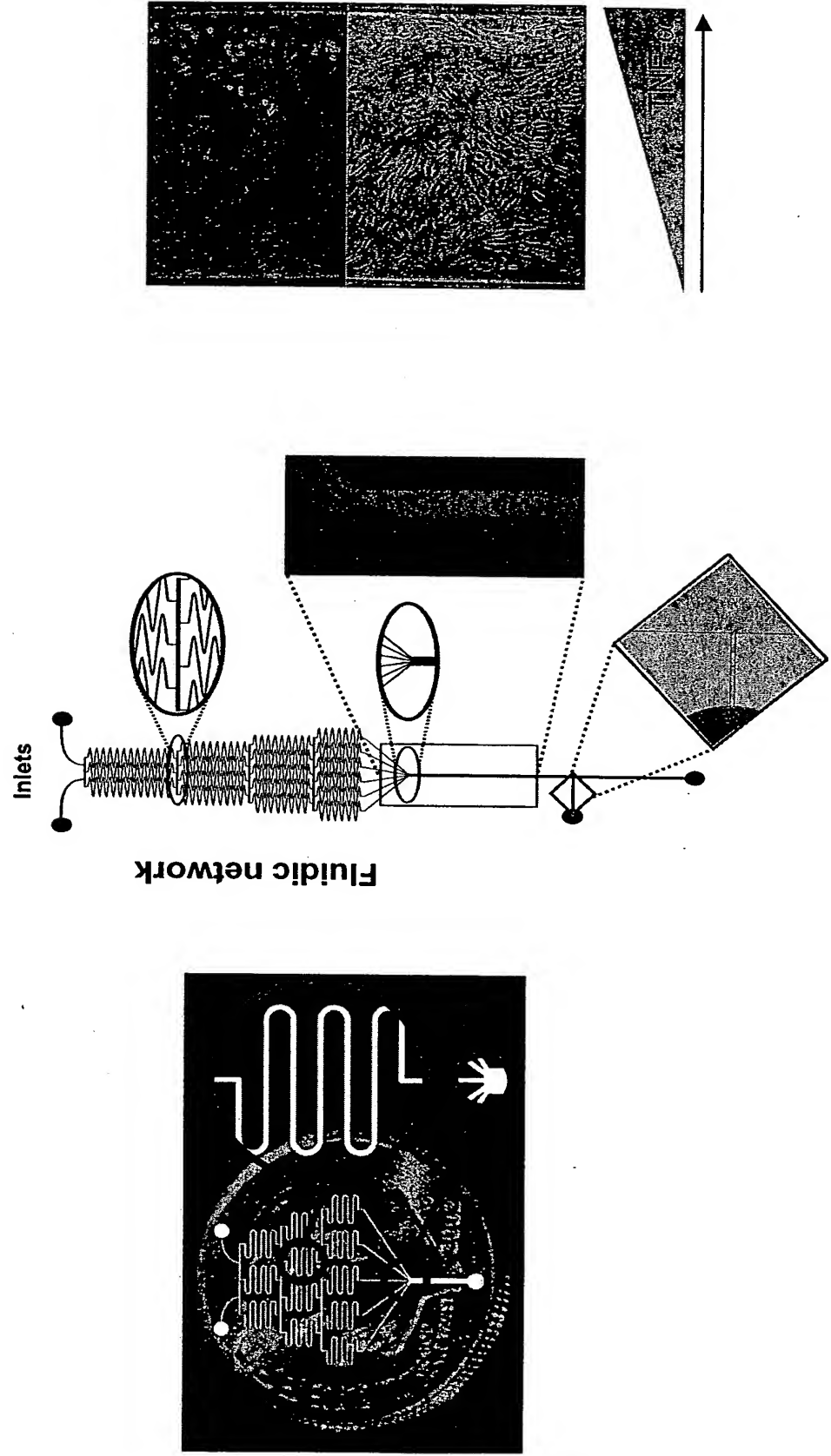
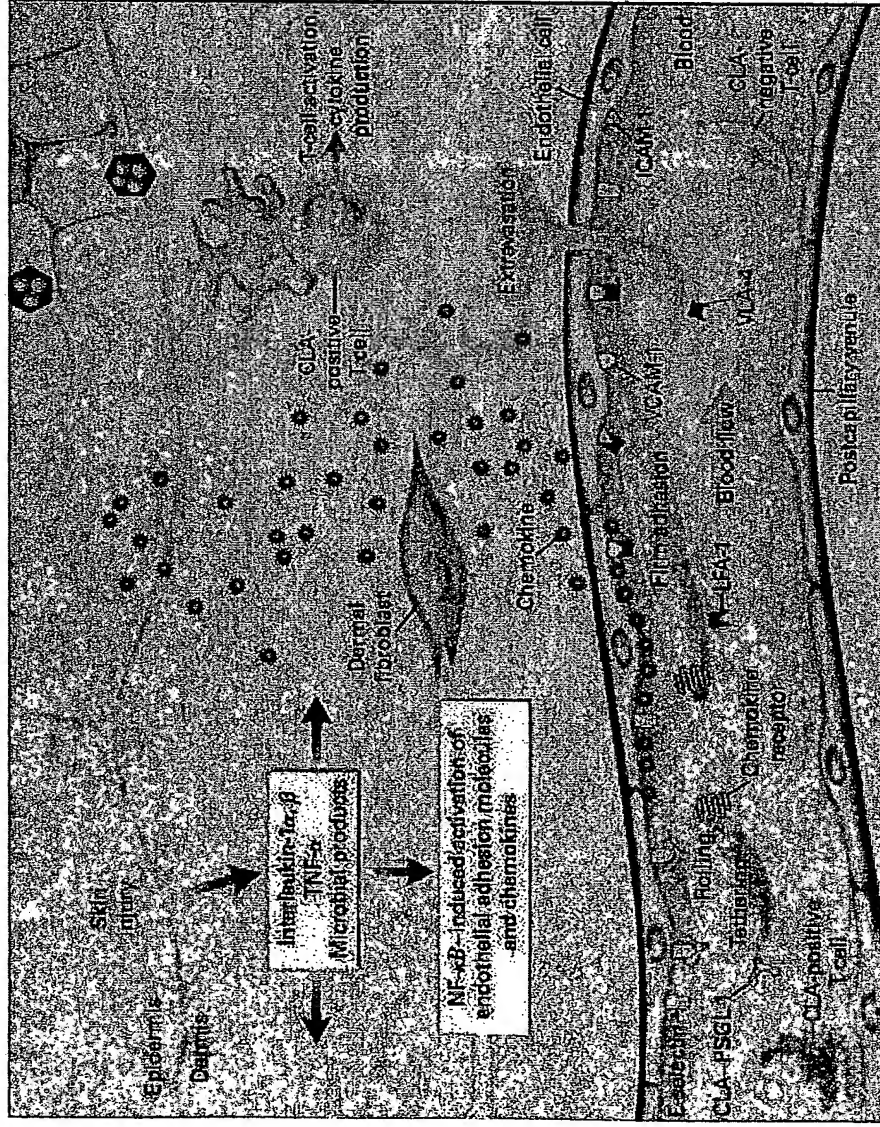


FIG. 95

Targeting Vascular Disease: Modeling Blood Flow (Gradients/Shear)



Inflammation Model

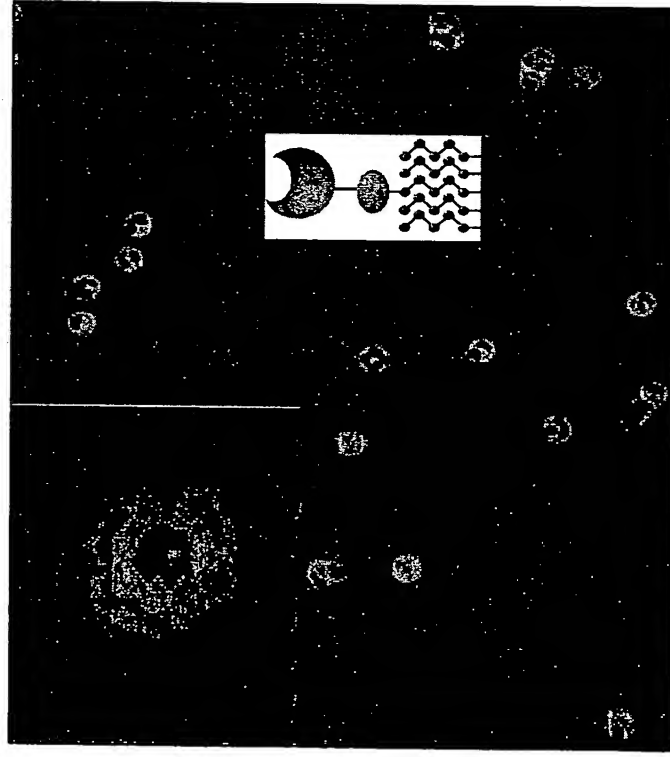


Assays

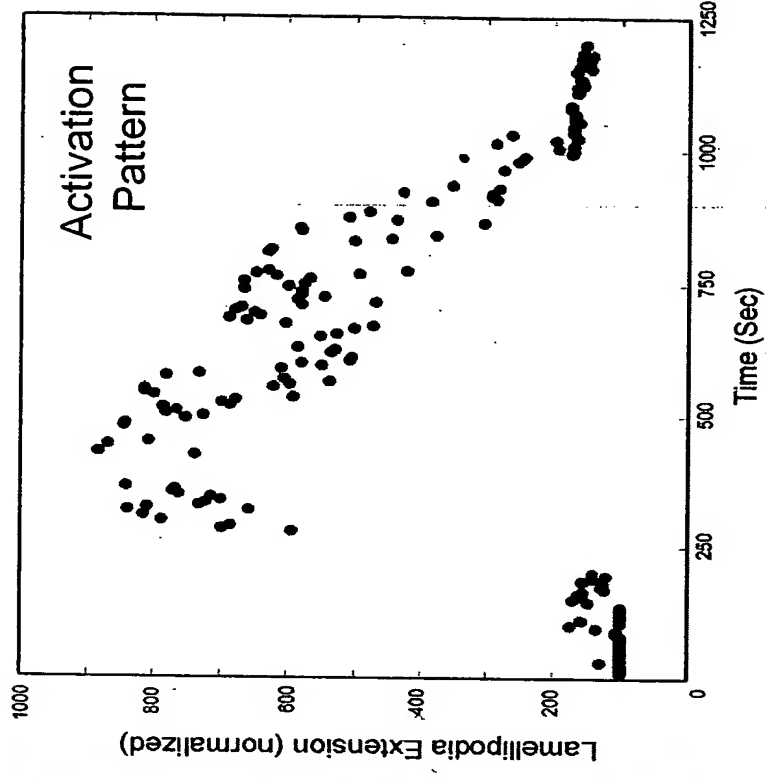
- Target biochemical characterization
- Endothelial cell activation
- Adhesion and Rolling of Leukocytes
- Transmigration of Leukocytes
- Chemotaxis of Leukocytes
- Immobilized Chemokine activation of Leukocyte
- Cell Motility

FIG. 97

Monocyte Activation- Morphology



Lamellipodia Extension
Time Lapse Video



Immobilized Chemokine

FIG. 98

Leukocyte Rolling and Adhesion on Endothelium

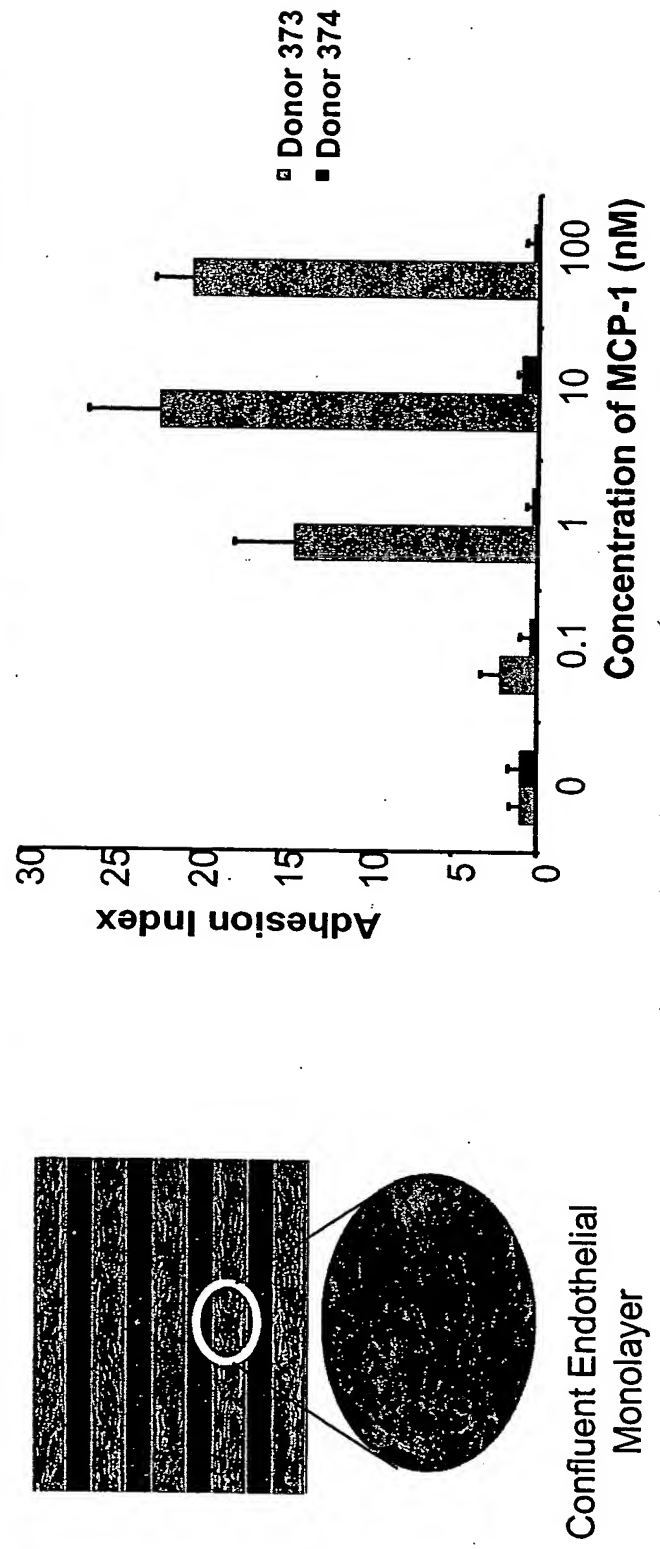
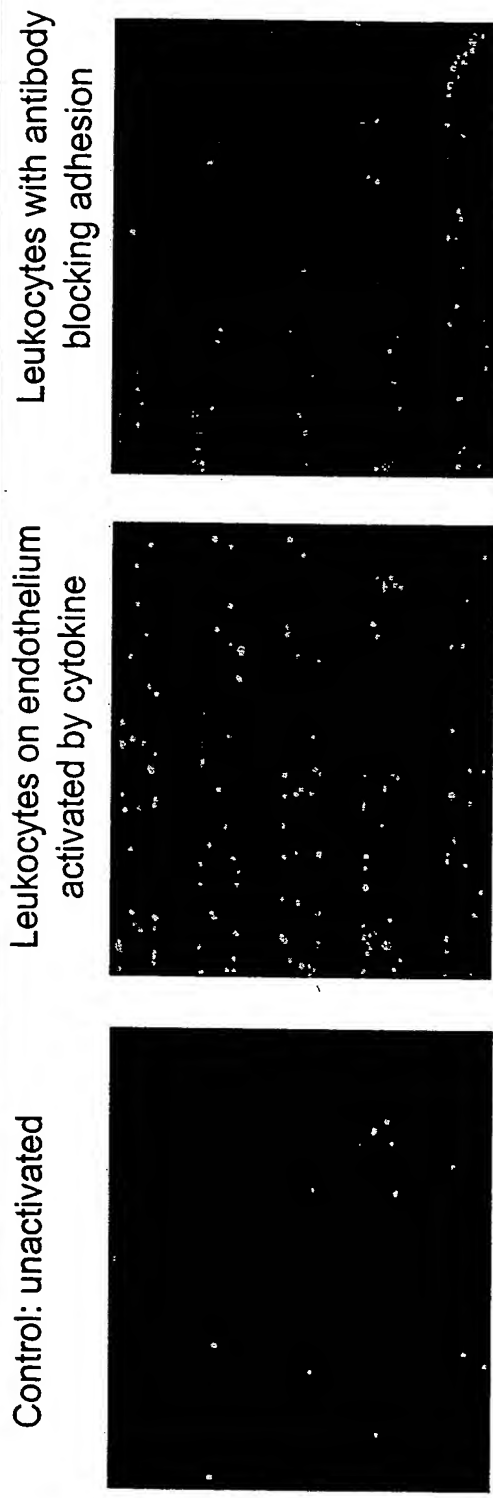


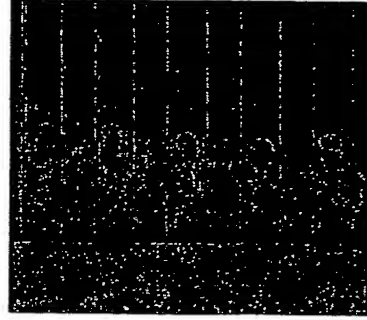
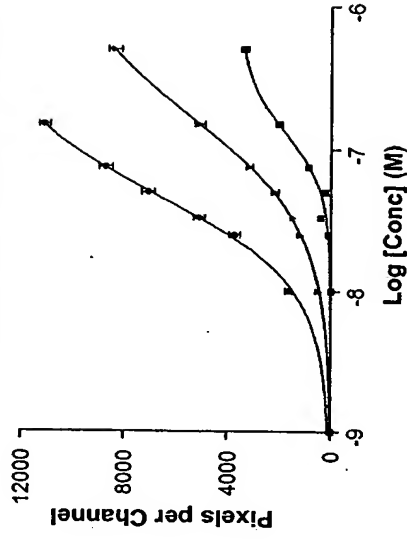
FIG. 99

Chemotaxis of Primary Monocytes

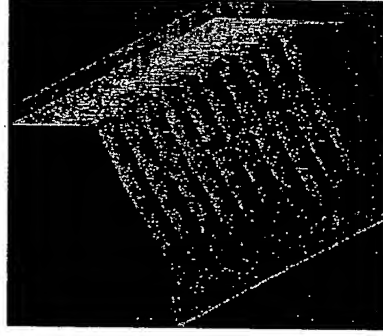
Morphological
characterization (with
chemokine gradients)



Triple Chemokine Curve



Spatial and temporal
readouts



Controlled matrix
and stable gradient

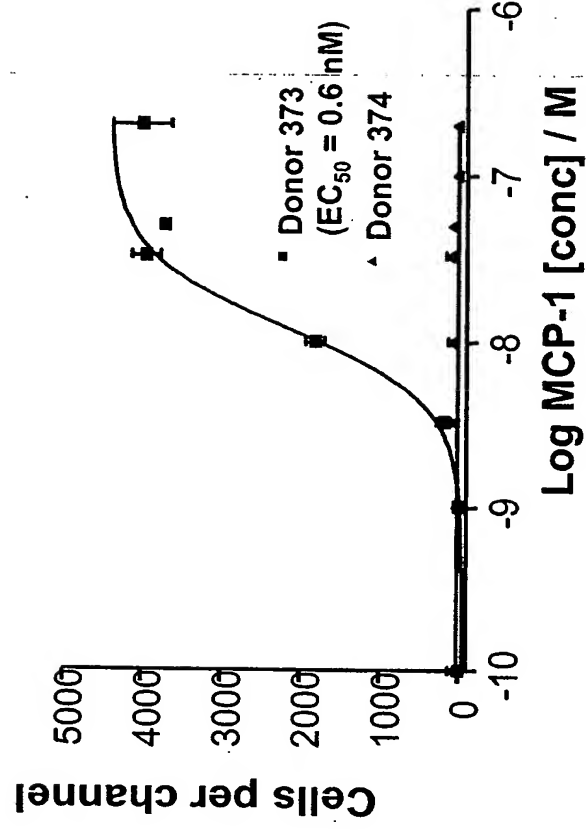


FIG. 100

Diapedesis- Monocytes (SLs 373 & 374)

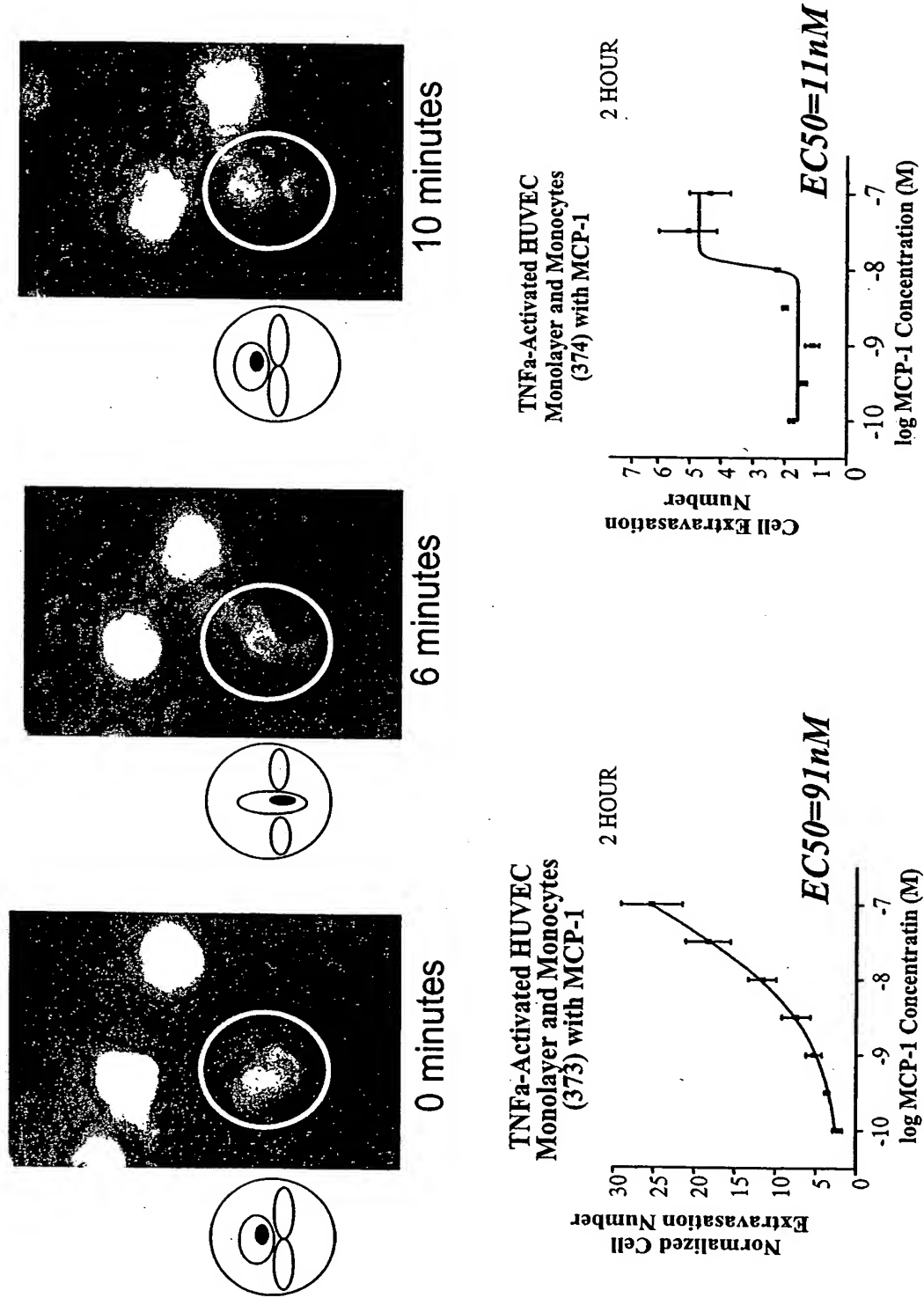
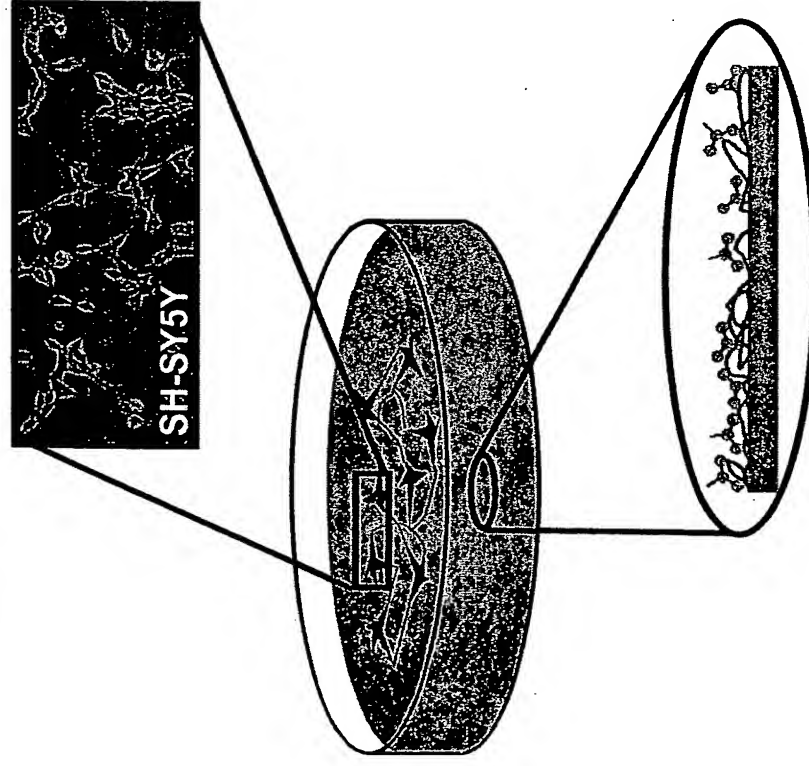


FIG. 101

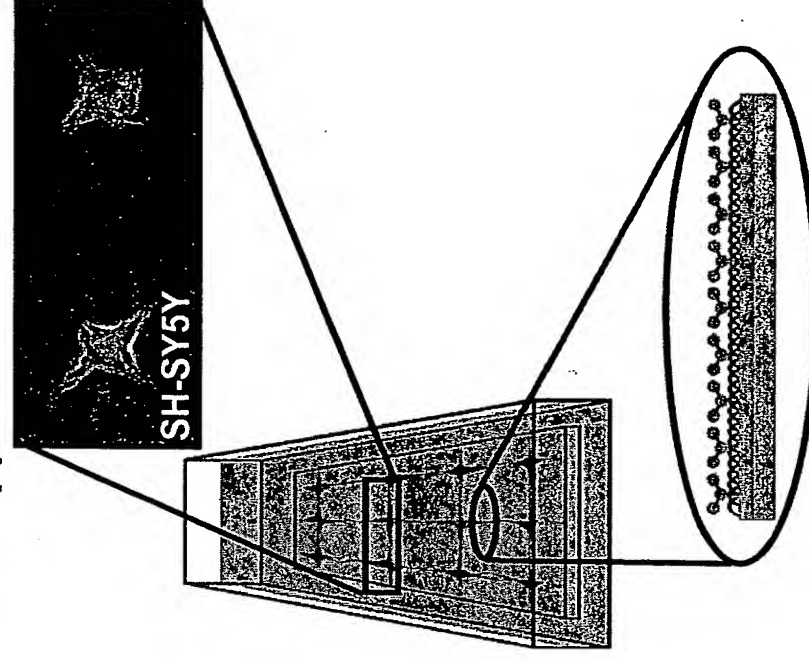
Other Disease Models: Controlled Microenvironments for Complex Cell Cultures

Conventional cell culture



- tissue culture dish
- media
- growth factor

SLx' approach to cell culture



- highly organized ECM-like surfaces
fibronectin, laminin, tenascin, collagen, GAGs...
- biological media
- fluidic delivery of growth factors
- predictable connectivity / architecture
- co-culture systems

Summary

Control cell physiology

Size and shape of a cell

Neighbouring cell types

The surfaces cells are attached to

The fluid environment they are exposed to

Control cellular micro-environments

Chemical parameters in 2-D and 3-D

Control of flow and other mechanical stimuli

Rapid modifications

Micro-environment compatible with low number of Primary Human Cells

Link and virtual subject profiles with compound response in the preclinical phase

*Ex-vivo Models of
Human Disease™*